GONADAL STEROID MODULATION OF OPIOID EFFECTS ON GONADOTROPIN SECRETION IN THE RAT

BY

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

GONADAL STEROID MODULATION OF OPIOID EFFECTS ON GONADOTROPIN SECRETION IN THE RAT

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The interactions between opiates and gonadal steroids in the control of the gonadotropins, luteinizing hormone (LH), and follicle stimulating hormone (FSH), were investigated in the rat. Male rats were chronically treated with morphine as a means of providing continuous opiate receptor stimulation. When initiated at the time of castration, both morphine treatment and testosterone (T) replacement prevented post-castration LH hypersecretion and hypothalamic LH-releasing hormone (LHRH) depletion. However, only T reversed these changes when treatments were initiated two weeks after orchidectomy. Further, in rats which had been castrated for

two weeks, morphine enhanced the ability of T to inhibit gonadotropin secretion and blocked the T-induced accumulation of LHRH in the hypothalamus. At physiological doses, it was found that 17-beta-estradiol (E2), but not 5-alpha-dihydrotestosterone (DHT), similarly interacted with morphine to suppress gonadotropin release. Further, the ability of morphine to interact with T in the suppression of LH release could not be explained by changes in hypothalamic norepinephrine, dopamine or serotonin metabolism.

Female rats were injected with the opiate antagonist, naloxone. Naloxone stimulated LH release at all times tested during the estrus cycle and following estradiol benzoate (EB) treatment to ovariectomized rats, indicating that endogenous opioid peptides (EOP) inhibit LH secretion throughout the estrus cycle, and during the proestrous and EB-induced LH surges. During LH hypersecretion, induced by progesterone (P) treatment to proestrous or EB-treated ovariectomized rats, naloxone was unable to stimulate LH release, indicating that EOP may contribute to the advanced onset and increased magnitude of the LH surge seen following P treatment.

In a final study, E2-treated ovariectomized rats were given morphine treatment. morphine enhanced both the negative and positive feedback effects of E2 on gonadotropin release in these animals. In summary, the work presented in this dissertation indicates that EOP play an important role

in the regulation of gonadal steroid feedback in male and female rats by modifying the sensitivity of the brain to circulating gonadal steroids.

CHAPTER I
INTRODUCTION

Opiates have been used by old world cultures for many centuries. Opium is the dried latex from the unripe seed pods of the plant Papaver somniferum. It was probably first discovered by the ancient Mesopotamians (Reynolds and Randal, 1957). The word 'opium' is derived from the Greek word for juice, and was mentioned by Theophrastus in the third century B.C. The Romans, Scribonius Largus and Galen both described the medicinal uses of opium. In another ancient reference to opium, the Kama Sutra states that Indian maidens and wives are forbidden to use opium in any form until after menopause, as the use of the drug prevents pregnancy (Kruger et al., 1941). Throughout the middle ages, Arabs used opium for medicinal purposes. They introduced the drug to China, where it was used to treat dysentery and other ailments. Its use in Europe increased during the sixteenth century, where opium was used for the treatment of diarrhea and pain, and as an adjunct to surgery.

Raw opium contains over 20 different alkaloids. Surturner isolated and described morphine in 1806 (Leake, 1975). Morphine comprises over 10% of the dry weight of opium. Its structure was proposed in 1925 by Gulland and Robinson, and

finally synthesized in 1952 by Gates and Tschudi (see Leake, 1975). Other less abundant alkalcids such as narceine, codeine, and thebaine were isolated in the early nineteenth century. Improved organic synthesis techniques in the twentieth century resulted in a flurry of semi-synthetic opiate and synthetic opiate-like compounds being produced. In an effort to design a less addictive opiate, the partial agonist, nalorphine, was introduced. Although analgesic and less addictive than morphine, its dysphoric properties precluded its widespread application. This search for a less addictive opiate also led to the synthesis of naloxone, an opiate antagonist relatively devoid of analgesic and other opiate agonist actions.

The modern use of opiates also led to its misuse. Great Britain secured a market for its Indian opium by addicting a large population in China (Kane, 1881). The disputes that developed between Great Britain and China over this trade were referred to as the Great Opium Wars of the eighteenth and nineteenth centuries. Chinese immigrants introduced opium smoking into the United States where it spread to the general population. Opiate abuse continued to increase in this country after the development of the hypodermic needle and the introduction of injectable morphine to relieve the pain resulting from battle injuries received during the Civil War (O'Donnell and Ball, 1966).

Many authors commented on opiate abuse at this time. In 1881, Kane described the physiclcgical cnsequences cf opiate abuse. Included in this discussion were the effects of opiate abuse on the sexual organs. Opium smokers exhibited "disgraceful cnduct and initially cnsiderable sexual stimulation, although the completion of the sex act was delayed". Several mnths cf abuse impaired bth desire and power. To further illustrate the decline in the sexual ability of opiate addicts, populatin statistics were cited. During the height cf the Great Cpium Wars, China's annual population increase was found tc fall frcm 6% to under 1%. It was even suggested that the cpium trade could be used as a means of controlling China's overpopulation!

The deleterious effects of cpiates and cther central nervous system depressants cn reproductive function continued to be reported in the medical literature during the twentieth century. While narcotic addicts appeared to commit fewer violent crimes such as rape (Finestone, 1957), men were found to be impotent and women showed inhibited sex drives and amenorrhea. The first contrclled clinical studies by Azizi et al. (1973) and Gaulden et al. (1964) reported depressed testosterone (T) levels in male hercin and methadone users and diminished sexual function in women narcotic addicts, respectively.

The numerous physical effects of cpiates were postulated to be the result of an interaction with specific central

nervous system receptors. In 1973, several laboratories independently reported the existence of opiate receptors in the nervous systems of mammals (Pert and Snyder, 1973; Terenius, 1973). Soon to follow these discoveries was the isolation of endogenous opioid peptides (EOP). Hughes et al. (1975) described the two pentapeptides, leucine-enkephalin and methionine-enkephalin, while Li and Chung (1976) reported the isolation of beta-endorphin. Following these discoveries, researchers began to identify the mechanisms through which opiates exerted their antigonadal effects. It soon became evident that opiates interfered with a normally operating EOP influence on reproductive hormone secretion. The effects of opiates on the regulation of reproductive hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH), is the subject of this dissertation.

CHAPTER II
REVIEW OF THE LITERATURE

This chapter will survey the major work with respect to the neuroendocrine control of gonadotropin secretion. This will include the basic anatomy and physiology of the hypothalamo-hypophyseal unit and neuropharmacological studies which contributed to the concept that an opioid inhibitory component controlling gonadotropin secretion. Emphasis will be placed on LH secretion, although important differences between LH and FSH secretion will be discussed. The field contains a wealth of literature and several reviews were employed as starting points and to highlight major trends and areas of agreement. This includes historical reviews by Garrisson (1929), Leake (1975) and Medvei (1982). The descriptions of general hypothalamo-hypophyseal anatomy were derived from Adams et al. (1965), Daniel (1966), Halasz (1969), Jenkins (1978), Ezrin (1979) and Palkovitz and Zaborsky (1979). Detailed reviews of the various neuronal systems can be found in Dahlstrom and Fuxe (1964), Fuxe and Understedt (1968), Cooper et al. (1978), Moore and Bloom (1978 and 1979), Sternberger and Hoffman (1978), Watson et al. (1980) and Palkovitz (1981). Information regarding luteinizing hormone releasing hormone

(LHRH) biochemistry, steroid concentrating neurons, and opioid peptides and their receptors may be found in reviews by Naftolin et al. (1975), Sar and Stumpf (1975), Bloom et al. (1978), McEwen et al. (1979), Childers (1980) and Martin (1981), Jutisz et al. (1983). Many excellent reviews concerning the role of monoaminergic neurons in regulating gonadotropin secretion have appeared over the years. Among the most complete are Coppola (1971), Weiner and Ganong (1978), Barraclough and Wise (1982), S. Kalra and Kalra (1983) and Simpkins et al., (1984). Finally, the pharmacological and physiological effects of opiate alkaloids and opioid peptides on gonadotropin secretion have been presented by Meites et al. (1979), Cicero (1980a and 1980b) and S. Kalra et al. (1980). While the literature discussed will focus primarily on studies in the rat, where appropriate, other animals will be discussed. The relation of these studies and the present work to the regulation of gonadotropin secretion in humans will be addressed in Chapter X.

Historical

Early Observations

Although modern endocrinology has yet to complete its first century, observations on endocrine function have been made throughout history. Relief carvings, figurines, and drawings from prehistoric, Egyptian, Babylonian, and later

Greek, Roman and Renissance artisans appear to depict medical conditions (Garrisson, 1929). Some of these artifacts have been interpreted as illustrating such illnesses as female endocrine system obesity, goiter and gigantisism. Goiter was endemic among ancient cultures and the Chinese prescribed an appropriate treatment of iodine-rich seaweed. While the Chinese, Hindus, and Egyptians all described diabetes melitis, the Greek Arctaec of Kappadckia coined the term 'diabetes' in the second century A.D.

Reproductive function was a primary concern of the ancients. The effects of castration on animals and humans have been known since prehistory, while the first ovariectomies in humans and hysterectomies on farm animals were performed by the Egyptions and Hebrews, respectively. Much later in the eighteenth century, the results of experimental removal of the gonads would be pioneered by John and William Hunter of England (Medvei, 1982).

The discovery of gynecological instruments among Roman ruins indicated some degree of medical sophistication during this period. Theories of reproduction were somewhat less advanced. The Hippocratic view that sperm arose from all tissues of the body to be stored in the testes was challenged by Aristotle who held that the right testes produced sperm destined to become males while the left testes produced sperm destined to become females. Additionally, Aristotle felt that the male provided all the

determining characteristics while the female provided merely a fertile enviroment for the development of the fetus.

A more modern view of the factors involved in reproduction awaited the development of the microscope in the seventeenth century. The initial works of Fallopic, deGraff, Leydig and many other scientists are reflected in our anatomical vocabulary. Leeuwenhock first described the presence of "little animals of the semen" in 1677 and 200 years later Hertwig demonstrated the union of the sperm and the ovum.

Recognition of the importance of the ovary in maintaining reproduction was the result of the work of many scientists. At the turn of this century, Walter Heap described the reproductive cycle in females and related the reproductive changes of the estrous cycle to those occuring in the menstrual cycle. The changes in vaginal cytology characteristic of the estrous cycle were first described by Long and Evans of America in the early twentieth century, while at the same time Hitschman and Adler of Vienna described the cyclical changes in the uterine endometrium which occur during the menstrual cycle.

The Development of Neuroendocrinology

The communication between various tissues of the body via substances released into the circulation is one of the central concepts of endocrinology. In the seventeenth and

eighteenth centuries Albrect von Haler and Frederick Buych recognized that the body contains ductless glands which release their contents into the blood. Claud Bernard refered to this process as internal secretion in 1855. In 1902, after his discovery of secretin, Ernest Starling coined the term 'hormone' to describe the active contents of internal secretions. Many hormones were first recognized by the ability of organ extracts to exert physiological effects on animals *in vivo* or effects on isolated tissues *in vitro*. Among the first hormones isolated were the gonadal steroids, testosterone (T), 17-beta-estradiol (E) and progesterone (P), which were found to be responsible for maintaining reproductive function in males and females.

One ductless organ which was found to be of major importance in maintaining normal homeostasis was the pituitary gland. Its function was long debated and, until 1838 when Rathke demonstrated the non-neural origin of the anterior portion, it was considered by many to be a vestigial portion of the brain. The pituitary gland was found to secrete substances necessary for normal body growth, the maintenance of reproduction, the initiation of lactation, and the restoration of atropied thyroid and adrenal tissue. In the 1940's C.H. Li and colleques isolated and synthesized the two gonadotropic hormones, luteinizing hormone and follicle stimulating hormone (LH and FSH, respectively; Li et al., 1940 and 1949).

A functional relationship between the brain and the pituitary gland was evident to many scientists. Galen's view that the pituitary drained phlegm from the brain to the nasopharnyx was refuted by the anatomist Schreiber in 1660. Schreiber's contemporary, Richard Lower proposed that substances from the ventricles perfused from the brain to the pituitary where they "percolated" into the circulation. This view is quite similar to our current views on neuroendocrine function.

In this century it was observed that many hypothalamic lesions and tumors disrupted endocrine function. Experiments showed that severing the pituitary connection with the brain atrophied both the thyroid and the adrenal glands. The transplantation of the pituitary gland to the renal capsule or the anterior chamber of the eye produced similar degeneration of the thyroid, adrenals and testes, but the ovarian corpera lutea was maintained in rats. It was apparent that the brain exerted both stimulatory and inhibitory influences on the pituitary. Additionally, this interaction between the brain and the pituitary seemed to be dependent on the preservation of the connection between the pituitary and the hypothalamus.

Joseph Lietaud first described the pituitary stalk and its relationship to the brain in 1742. It was not until 1930 that Popa and Fielding clearly described the vascular link between the two organs as a portal system, however. It is

unfortunate that the two scientist incorrectly proposed that the direction of blood flow in the portal system was from the pituitary to the hypothalamus Housay el al. (1935) and Wislocki and King (1936) quickly rectified this error.

Several observations led to the development of our current understanding of neuroendocrine relationships. Among the foremost was the discovery of neurosecretion by magnocellular neurons in the paraventricular and supraoptic nuclei by Scharrer and Scharrer (1940). These neurons released oxytocin and vasopressin from nerve terminals in the neural lobe of the pituitary into the general circulation. Harris and associates performed numerous studies which demonstrated the importance of the hypothalamus in regulating anterior pituitary function. These included endocrine changes following the electical stimulation of the hypothalamus. In 1948, Harris proposed the "chemoreceptor hypothesis" to explain the control of anterior pituitary function by hypothalamic hormones released into the portal circulation. This concept remains one of the cornerstones of neuroendocrine thought.

Much of the work of neuroendocrinologists over the past 30 years has concerned the demonstration, isolation and characterization of these hypothalamic hormones. Halasz et al., (1962) used the term hypophysiotropic area to describe the regions of the hypothalamus that would support pituitary grafts. Using <u>in vitro</u> assays, releasing activity was

demonstrated in hypothalamic extracts for thyroid stimulating hormone (Shibusawa et al., 1956), LH (McCann et al., 1960), prolactin (Meites et al., 1960), FSH (Igarshi and McCann, 1964; Mittler and Meites, 1964), and growth hormone (Deuben and Meites, 1964). Hypothalamic release inhibiting activity was shown for prolactin (Talwalker et al., 1961; Pasteels, 1961) and growth hormone (Krulich et al, 1968).

The isolation of these releasing factors proved to be more difficult. Because these factors were present in very small amounts, sensitive biochemical and bioassay techniques as well as large amounts of tissue were necessary. Many incorrect claims have been put forth over the years. The first releasing factor successfully isolated was the tripeptide, thyrotropin releasing hormone. It was found to stimulate both prolactin and thyroid stimulating hormone release and its structure was simultaneously reported in the laboratories of Andrew Schally and Roger Guillemin (Scahlly et al., 1969; Burgus et al., 1969). Schally's group also described the next releasing factor. It was a decapeptide which stimulates the release of both LH and FSH, but because other factors appear to also regulate FSH release, it has been termed luteinzing hormone releasing hormone (LHRH, Martsuo et al., 1971). The competition to isolate hypothalamic releasing factors continued as Guillemin's laboratory reported the sequence of a growth hormone

release-inhibiting factor, now called somatostatin (Brazeau et al. 1973). The rivalry between these two laboratories did not go unrecognized in the scientific community. In 1977, together with Rosalyn Yallow, Roger Guillemin and Andrew Schally received the Nobel Prize in Physiology. The drive to isolate releasing factors continues today. Corticotropin releasing factor was isolated by Wylie Vale, Guillemin's colleague at the Saulk Institute (Vale et al., 1981). In the following year Vale and Guillemin simultaneously reported the sequence for a growth hormone releasing factor (Guillemin et al., 1982, J. Rivier et al., 1982)

Neuroanatomical Relationships

Anatomy of the Pituitary Gland

The pituitary gland lies within a portion of the sphenoid bone called the sella tursica. It is positioned below the mid-ventral portion of the brain and is encased in an extension of the cerebral meninges known as the sellar diaphragm. The two major anatomical divisions of the pituitary gland, the neurohypophysis or posterior pituitary and the adenohypophysis or anterior pituitary, have distinct embryological origins. The neurohypophysis is derived from neural ectoderm and contains the nerve terminals of magnocellular neurons of the hypothalamus. It is involved in the neurosecretion of vasopressin and oxytocin. The adenohypophysis is derived from an envagination of the

stromal ectoderm called Rathke's pouch and has no direct neural connection with the brain. It constitutes 80% of the weight of the pituitary gland. Another less prominent division of the pituitary gland is the intermediate lobe. While derived from Rathke's pouch, it does receive innervation from the hypothalamus and is often included in dissections with the neurohypophysis. Together these two lobes are termed the neurointermediary lobes (NIL). While distinct in the rat, the intermediate lobe is only well developed in humans during pregnancy.

The major anatomical division of the adenohypophysis is the pars distalis. Approximately 5% of the cells of the pars distalis are gonadotropin producing cells. It is still uncertain whether a separate type of gondadotroph exists of LH and FSH. There is evidence that some endocrine manipulations, such as ovariectomy, produce two distinct populations of gonadotropes.

General Hypothalamic Anatomy

The hypothalamus constitutes the ventral portion of the diencephalon. Its many neural connections with other portions of the diencephalon, limbic system and brainstem highlight its important role in endocrine and autonomic homeostasis. The boundaries of the hypothalamus are defined as the ventral surface of the brain extending from the rostral border of the optic chiasm to the mammillary bodies

caudally, and laterally to the hippocampal sulcus and optic tracts. The dorsal border of the hypothalamus is recognized as the anterior commissure and lamina terminalis rostrally to the hypothalamic sulcus and cerbral aqueduct caudally. In rats the hypothalamus constitutes 1% of the weight of the brain.

An important landmark evident from the midventral surface of the brain is a prominence containing the infundibulum and tuber cinerium which together constitute the median eminence. This is the sole anatomical connection between the hypothalamus and the pituitary gland. A unique vascular system supplies this hypothalamo-hypophyseal unit. Hypophyseal arteries branch off the Circle of Willis to perfuse the various hypothalamic regions. The most important aspect of the hypothalamic circulation is the vascular supply to the median eminence and arcuate nucleus. Arteries in this area form a capillary network containing multiple anastomoses which is refered to as the primary plexus or palisadic zone. A portion of this configuration contains numerous nerve endings bordering these vascular spaces and is called the external layer of the median eminence. Because these capillaries do not contain fenestrations, the median eminence is not included in the blood brain barrier and is considered one of the brain's circumventricular organs.

The veins of the median eminence collect to form a portal system which supplies blood to a secondary capillary plexus in the pituitary. Venous flow from the pituitary is achieved through sinuses adjacent to the adenohypophysis. This hypothalamo-hypophyseal portal system is the primary blood supply to the pituitary gland. Its existence provides a means for neurosecretory products of the hypothalamus to reach the adenohypophysis in high concentrations undiluted by the general circulation. While the direction of blood flow is from the hypothalamus to the pituitary, this system may be more complex than previously thought. Bergland and Page (1979) have proposed several other pathways through which elements in this portal system may interact.

Cell bodies of the hypothalamus are distributed in three major gray regions, the anterior, intermediate, and posterior areas. The anterior and intermediate regions are the most important in the regulation of anterior pituitary hormone secretion. Hypothalamic nuclei are generally located bilaterally on either side of the third ventricle. The exceptions to this rule are the arcuate nucleus and its closely associated median eminence which are located at the ventral border of the third ventricle surrounding the infundibular recess. The anterior hypothalamic area includes the preoptic area located rostral to the optic chiasm. Consequently this area is often collectively refered to as the preoptic area-anterior hypothalamus (POA-AH). Important

nuclei in this region include the suprachiasmatic nucleus which is immediately dorsal to the optic chiasm, the anterior hypothalamic nucleus located dorsalateral to the suprachiasmatic nucleus, the paraventricular nucleus which constitutes the rostral preoptic area to the dorsalateral wall of the third ventricle, the supraoptic nucleus, and the organum vasculosum of the lamina terminalis which is a circumventricular organ located at the anterior ventral third ventricle. The preoptic and and supraoptic nuclei appear to be particularly important in the cyclic release of gonadotropins in the rat, but not necessarily in the human (Hillarp, 1949, Halasz, 1969, and Krey et al., 1975).

The intermediate and posterior areas are often grouped into a tissue section refered to as the medial basal hypothalamus (MBH). Nuclei of the intermediate hypothalalamus include the lateral hypothalamic nucleus, ventralateral nucleus, dorsalmedial nucleus and the arcuate and median eminence nuclei. Lesions of the median eminence or arcuate nucleus disrupt gonadotropin secretion and result in a loss of reproductive cycles (Goodman and Knobil, 1981).

The nuclei of the hypothalamus have numerous afferent and efferent connections. Seven afferent tracts impinge upon the hypothalamus. The medial forebrain bundle contains tracts originating in both the olfactory cortex and the brainstem while the stria terminalis originates in the amygdala. Both tracts terminate in the hypothalamus,

midbrain, anterior commissure and preoptic area. Corticomedullary fibers include the fornix and corticchypothalamic tracts which originate in the hippocampus and frontal cortex, respectively. Afferents from the medial thalamic nuclei and subthalamus reach the hypothalamus via the periventricular regions. The mammillary bodies receive inputs from the ascending spinal tracts, the brainstem and the anterior thalamic nucleus. Finally, photic input to the suprachiasmatic nucleus is received via a direct retino-hypothalamic tract.

Several efferent pathways have been described as originating in the hypothalamus. Two tracts, the supraoptico-hypophyseal and the tubero-hypophyseal, terminate in the NIL. The fasciculus mammillary princeps terminate in the anterior thalamus and midbrain. A periventricular fiber system returns inputs to the midbrain thalamic nuclei from the posterior, tuberal and supraoptic hypothalamic areas. While the primary direction of all these pathways is either afferent or efferent, it is not absolute. In addition, many smaller pathways cannot be identified without immunocytochemical techniques. The relation of these tracts as well as intrahypothalamic pathways to the neurotransmitter systems will be discussed in the following sections.

Anatomy of the Luteinizing Hormone-Releasing Hormone Neuronal Systems

Many problems associated with immunocytochemical techniques have precluded a complete description of the distribution of LHRH neurons. Among these problems is the lack of an amino acid sequence for a LHRH precursor or its mRNA sequence which could be used to visualize LHRH-containing perikaryia. Many antibodies to LHRH are conformationally restricted or require one or both terminal ends of the LHRH molecule for recognition. This can prevent the visualization of peptide-containing cell bodies. To demonstrate LHRH in neuronal perikaryia many studies have employed cholchicine or barbiturate pretreatment, or deafferentation (Sternberger and Hoffman, 1978).

Several generalizations may be made regarding the distribution of LHRH neurons in the rodent brain. The neurons are bipolar or fusiform and have distributions not limited to the traditional nuclear boundaries of the hypothalamus. Nerve terminals are heavily concentrated in the external layer of the median eminence, which fits the role of LHRH as a hypothalamic releasing hormone. At least two distinct neuronal pathways appear to exist:

1. a tuberoinfundibular pathway with cell bodies in the arcuate nucleus projecting to the median eminence.
2. a preoptico-tuberal pathway with cell bodies in the medial preoptic zone that project to the median eminence.

Although LHRH perikaryia have been visualized in the arcuate nucleus of several species, many groups have had difficulty demonstrating their presence in the rat (Sternberger and Hoffman, 1978). However, deafferentation spares 20% to 30% of LHRH concentrations in the MEH and does not disrupt basal LH secretion (Blake and Sawyer, 1974; S. Kalra, 1976; Brownstein et al., 1977; Sopor and Weik, 1980). Since this does not seem to be due to incomplete lesions, there appears to be some LHRH neurons inside the area of the cut.

A recent study by Kelly et al. (1982) reports the presence of LHRH perikaryia in the MEH, especially the lateral arcuate nucleus and median eminence. This study employed a specific LHRH antisera which was not conformationally restricted, and used rigorous fixation procedures on saggital brain sections to facilitate the visualization of the cell bodies. These neurons and others located in the retrochiasmatic nucleus, preoptic area, and organum vasculosum of the lamina terminalis, terminated in the external layer of the median eminence.

Other recent studies have expanded the potential distribution of LHRH cell bodies in the brain. These include septal projections and preoptic projections to the organum vasculosum of the lamina terminalis, the olfactory bulb and midbrain central gray (Dluzen and Ramerez, 1981; Witkin et al., 1982; Shivers et al., 1983b).

Anatomy of the Monoaminergic Neuronal Systems

The gradual improvement of analytical procedures has resulted in a fairly accurate description of the distribution of monoaminergic neurons and their terminal beds in the central nervous system. Concentrations of the monoamines can also be determined in small nuclear regions using the "punch technique" of Palkovitz (1981). In addition, with the use of antibodies generated against catecholamine synthetic enzymes such as phenylethanolamine-n-methyltransferase, which converts norepinephrine (NE) to epinephrine (EPI), these two neuronal systems can be more easily differentiated.

Noradrenergic pathways

Voogt (1954) first demonstrated the presence of NE in the brain. It now appears that the hypothalamus receives a rich afferent innervation from NE-containing neurons. Evidence favoring an external source for NE in the hypothalamus includes the depletion of 70% to 90% of hypothalamic NE after complete deafferentation (Weiner et al., 1972). The residual NE seen after deafferentation or brainstem lesions appears to be due to glial cells concentrating NE and to collateral reinnervation, since no NE-containing perikaryia have been visualized in the hypothalamus (Palkovitz, 1981).

Noradrenergic neurons are clustered in 5 major cell groups in the brainstem - the lateral reticular nucleus, the

solitary tract nucleus, the ventral pontine nucleus, the locus ceruleus, and the mesencephalic reticular formation. These cell groups innervate the hypothalamus through three major tracts - the ventral noradrenergic bundle, and the ventral and dorsal periventricular noradrenergic bundles. The ventral noradrenergic bundle joins the medial forebrain bundle prior to innervating the hypothalamus and appears to be the most important of the three tracts in regulating anterior pituitary hormone secretion. Although cell bodies that contribute to the ventral noradrenergic bundle are primarily located in the lateral reticular nucleus, the interconnections between all the various cell groups and their ability to form collateralizations must be stressed.

Dopaminergic pathways

Several distinct dopaminergic pathways are found in the brain. The largest consists of dopamine (DA)-containing neurons in the zona compacta of the substantia nigra and ventral tegmentum. From these neurons arise the nigralstriatal and mesocortical-mesolimbic DA systems. However, neither of these systems appear to innervate the hypothalamus significantly (Weiner et al., 1972).

The DA innervation of the hypothalamus arises primarily from intrahypothalamic DA systems. The tuberoinfundibular DA system consists of cell bodies in the arcuate nucleus which project to the median eminence. Axon collaterals terminate

in the arcuate nucleus, ventralateral nucleus and premammillary nuclei. Included in this system is the tuberohypophyseal DA tract which contains cell bodies in the arcuate and periventricular nuclei that project to the neurohypophysis and intermediate lobes of the pituitary (Moore and Bloom, 1978; Palkovitz, 1981). Lastly, a poorly defined collection of DA-containing neurons in the zona incerta, dorsalmedial subthalamus and posterior hypothalamus form the incerto-hypothalamic DA system. These cells project to the dorsal anterior hypothalamus, and the paraventricular and lateral septal nuclei (Bjorklund et al., 1975).

Epinephrine-containing pathways

Of the three major catecholamines, EPI has the narrowest distribution. Two EPI-containing cell groups are intermingled with NE-containing cells in the lateral tegmentum and dorsal medulla. In addition to other areas, these cells project to the hypothalamus via the medial forebrain bundle (Moore and Bloom, 1979; Palkovitz, 1981).

Serotonergic Pathways

There appears to be a widespread distribution of serotonin (5HT) terminals throughout the brain. Clusters of 5HT-containing perikaryia are restricted to the midline brainstem raphe nuclei. Ascending fibers course through the medial forebrain bundle and innervate the hypothalamus,

particularly the suprachiasmatic nucleus (Kuhar et al., 1972). In addition, there is evidence for 5HT perikaryia within the hypothalamus (Fuxe and Understedt, 1968).

Anatomy of Endogenous Opioid Peptide Containing Neuronal Systems

At present three distinct families of endogenous opioid peptides (EOP) have been characterized. Each appears to have its own unique precursor molecule, anatomical distribution and receptor subtypes. Because of the difficulties in producing specific antibodies which distinguish between these three groups of molecules and in visualizing peptide-containing cell bodies, the description of EOP neuronal systems is far from complete.

Beta-endorphin-containing neurons

Beta-endorphin is derived from proopiomelanocortin (POMC) and has been colocalized in cells with other POMC-derived molecules like adrenocorticotropin, melanocyte stimulating hormone, and beta-lipotropin (Mains et al., 1977). It is not clear whether all POMC-containing neurons release the same degradation products, or whether as Watson et al. (1980) suggest, several distinct POMC-neuronal populations exist, each releasing a characteristic set of molecules. One clearly defined beta-endorphin pathway is agreed upon (Bloom et al., 1978; Finley et al., 1981a). Fusiform neurons are contained in the tuberal hypothalamus extending from the

lateral arcuate nucleus to the lateral hypothalamic border. Fibers project to the anterior hypothalamus and septum where the pathway reverses direction and follows the stria terminalis to terminate in the dorsal raphe, locus ceruleus and central gray. Arborizations are found throughout the POA-AH and MBH including the median eminence and arcuate nucleus. Electronmicroscopic studies have revealed local interactions between POMC-containing neurons in the hypothalamus (Kiss and Williams, 1983).

Enkephalin-containing neurons

Enkephalins are derived from a precursor molecule which contains methionine-enkephalin, carboxy-terminal extended methionine-enkephalin and leucine-enkephalin. While beta-endorphin-containing neurons have a fairly distinct pathway, enkephalin-containing neurons are widely distributed throughout the brain. The highest concentrations of methionine-enkephalin are found in the striatum, followed by the hypothalamus. Dense methionine-enkephalin innervation has been found in the external layer of the median eminence (Watson et al., 1980). Enkephalin-containing cells are generally interneurons although several short pathways have been described.

1. A dense collection of methionine-enkephalin-containing cell bodies in the central amygdala project to the bed nucleus of the stria terminalis (Cuello and Paximus, 1978).

2. Magnocellular neurons of the paraventricular nuclei contain methionine-enkephalin and project to the neurohypophysis (Rossier et al., 1979).
3. A preopticotuberal pathway with cell bodies in the POA-AH projects to the median eminence (Finley et al., 1981b).
4. Methionine-enkephalin-containing perikeryia in the anterior hypothalamus terminate in the septal area (Sakanaka et al., 1982).

Dynorphin-containing neurons

Dynorphin is derived from the prodynorphin-alpha-neoendorphin precursor along with leucine-enkephalin and carboxy-terminal extended leucine-enkephalin molecules (Goldstein et al., 1979; Kakadani et al., 1982). Consequently, there is some difficulty in distinguishing between leucine-enkephalin contained within prodynorphin- and proenkephalin-containing neurons. Of the three families of EOP, the distribution of dynorphin-containing neurons is the most restricted. A supraoptico-neurohypophyseal pathway appears to be distinct from a similar methionine-enkephalin containing pathway. Some of these supraoptic neurons also contain vasopressin and corticotropin releasing factor and project to the median eminence in addition to the neurohypophysis (Watson et al., 1982a,b; Roth et al., 1983).

In addition to the brain, EOP are found in the pituitary gland. Beta-endorphin coexists with other POMC molecules in adenohypophyseal corticotropes and is released into the blood by stimuli which also stimulate adrenocorticotropin secretion (C. Rivier et al., 1982). The anterior pituitary and intermediate lobes contains some of the highest concentrations of methionine-enkephalin in the body (Bloom et al., 1977; Kumar et al., 1979). Because of the presence of EOP and their receptors in the pituitary, their potential influence on anterior pituitary hormone release cannot be discounted (Simantov and Snyder, 1978).

Steroid Concentrating Neurons in the Brain

While it is recognized that neurons may serve a target sites for gonadal steroids, the identities of these steroid concentrating neurons are not known. Specific cytoplasmic and nuclear receptors for androgens, estrogens, and progestins as well as the steroid metabolic enzymes 5-alpha-reductase and aromatase have been characterized in the brain and hypothalamus (Massa et al., 1972; Naftolin et al., 1975; Sar and Stumpf, 1975; McEwen et al., 1979). The electrical activity and morphological characteristics of neurons in the hypothalamus can be altered by estrogens (Pfaff and McEwen, 1983; Toran-Allerand et al., 1983).

Neuroanatomical Interactions

Although the distributions of LHRH, monoamine, and ECF-containing neurons show considerable overlap, evidence for a direct anatomical interaction between these systems in the hypothalamus is lacking. The anatomical relationships between peptide-containing and noradrenergic neurons have been most intensely studied. Methionine-enkephalin containing nerve terminals do appear to synapse on catecholamine perikaryia and axons (Iegar et al., 1983; Schwartz, 1979). While reports of such an interaction in th hypothalamus are lacking, Hoffman et al. (1982) presented evidence for noradrenergic terminals synapsing with LHRH cell bodies en passant. Considering the close proximity of many nerve terminals in the median eminence, a diffuse nonsynaptic interaction between any of these systems is possible. This is particularly reasonable considering the extended half-life of beta-endorphin (Bloom et al., 1978), which would allow its diffusion to adjacent nerve terminals even in the absence of classical synaptic contacts.

Improved anatomical methods may further resolve the neuroanatomical relationships in the hypothalamus. Ronkleiv et al. (1981) compared beta-endorphin and LHRH immunoreactivity in adjacent brain slices and found beta-endorphin containing neurons and terminals to be more widely distributed than those containing LHRH in the MBH. Double immunostaining procedures have not been employed. By

comparing autoradiography and immunocytochemistry Shivers et al. (1983a) reported that LHRH neurons do not appear to concentrate estradiol in their nuclear regions. This contrasts with the report that some neurons in the hypothalamus that are electrically sensitive to estrogen do stain for LHRH (Kelly and Ronkleiv, 1982).

Patterns of Gonadotropin Secretion

Gonadotropin secretion is inherently pulsatile. Whether absolute levels of LH and FSH are determined at a single time point across several animals or within a single animal over time, these levels represent the summation of pulsatile discharges of hormone which vary in frequency and amplitude. Because of the longer half-life of FSH (Coble et al., 1969) pulsatile LH secretio is most frequently studied.

Males

In the male, LH secretion is characterized by hourly low amplitude pulses which are temporally related to periodic T episodes (Ellis and Desjardins, 1982). Although LH levels remain fairly constant throughout the day, T levels are highest in the midafternoon and lowest at about midnight. Peak levels of P and FSH coincide with midnight, while LHRH concentrations in the MBH fluctuate showing lowest levels between 1100 h and 1600 h and peak concentrations at 1900 h through 0800 h (P. Kalra and Kalra, 1977b).

The removal of gonadal steroid feedback by gonadectomy is followed by marked changes in the hypothalamic-pituitary-LH axis. Within hours of gonadectomy in the male LH secretion is increased, and by two weeks after castration displays characteristic high amplitude pulses with a frequency of 20 to 30 minutes (Badger et al., 1978; Gallo, 1980a). Concentrations of LHRH decline in the MBH while the pituitary responsiveness to LHRH and receptors for the decapeptide increase (P. Kalra and Kalra, 1977; Nansel et al., 1979; Conne et al., 1982). All of these effects are reversed by T.

While FSH secretion shows a similar response to castration, T replacement is much less effective in returning FSH to gonadal-intact levels (Mahesh et al., 1975). This is in agreement with reports that other gonadal and hypothalamic factors also regulate FSH secretion (McCann et al., 1983).

Females

In the female rat low levels of gonadotropin secretion are interrupted by a preovulatory discharge of LH and FSH every 4 to 5 days. Gonadotropin levels during the proestrous surge may be 5 to 20 times greater than basal levels. Whether basal or surging, LH and FSH levels in the female rat are the result of pulsatile discharges of hormone (Gallo, 1981a,b). Prior to the proestrous gonadotropin

surge is a period of follicular development characterized by increasing estrogen titers (P. Kalra and Kalra, 1977a). These sustained estrogen levels permit the expression of a daily signal for LH release to be timed to the midafternoon (Legan et al., 1975). Superimposed upon this cyclic pattern of LH and estrogen secretion is a circadian variation in P secretion by the adrenal and ovary. Peak P levels occur in the evening prior to midnight (S. Kalra and Kalra, 1974a). The largest of these P rhythms is on proestrus, when it may contribute to the preovulatory release of gonadotropins.

This proestrous LH surge is preceded by a period of LHRH accumulation in the MBH and accompanied by a decline in levels of the decapeptide (S. Kalra and Kalra, 1981). The decline in MBH LHRH levels appears to be an indication of enhanced LHRH release (Sarkar et al., 1976). Also accompanying this preovulatory interval is a marked increase in the LH secretory response to LHRH and an increase in pituitary LHRH binding sites (Cooper et al., 1973; Aiyer et al., 1974; Savoy-Moore et al., 1980).

When the feedback effects of gonadal steroids are removed as a result of ovariectomy, the response of the pituitary to LHRH immediately increases (Cooper et al., 1975). Luteinizing hormone levels gradually increase over a three week period, the result of increased LH pulse amplitude and a small increase in LH pulse frequency (Weick et al., 1981).

The reinitiation of negative and positive feedback in ovariectomized rats by gonadal steroid treatment can serve as a controlled method for studying gonadotropin secretion. Immediately after the injection of estrogen or the implantation of estrogen containing capsules to ovariectomized rats, LH levels decline and the pituitary becomes refractory to LHRH (Vilchez-Martinez et al., 1974). Two days of continuous estrogen exposure enhances the response of the pituitary to LHRH and induces a diffuse midafternoon LH surge. This afternoon LH surge repeats for several days if estrogen titers remain elevated (Legan et al., 1975)

The injection of P several hours before the onset of the LH surge further increases the pituitary response to LHRH, enhances the magnitude of the resultant LH surge, and advances its onset (Aiyer et al., 1976; Kalra et al., 1981). Concentrations of LHRH in the MBH increase prior to and decline during the period of LH hypersecretion in a fashion similar to that seen on proestrus (S. Kalra and Kalra, 1979).

Monoaminergic Control of Gonadotropin Secretion

Of the major neurotransmitters, the influence of the monoamines in controlling gonadotropin secretion has been most extensively studied. These neurotransmitters are also important in EOP regulation of hormone output. The following

section will briefly review our current understanding of monoaminergic regulation of LH and FSH secretion. While many trends are apparent in this area, it must be cautioned that few monoaminergic drugs are specific for one neurotransmitter or receptor. Rather, it is the integration of many studies and confirmations of those works that provide an accurate description of the role each neurotransmitter plays in the control of gonadotropin release.

Many studies have evaluated the activity of monoaminergic neurons in various reproductive states, especially the catecholamine neurons (Weiner, 1974; Cooper et al., 1978). Methods of estimating catecholamine neuronal activity which do not disturb the steady-state include the rates of synthesis of NE or DA from trace amounts of their radiolabeled precursor, tyrosine, or the disappearance of trace amounts of added radiolabeled catecholamine. This technique can also be employed for the evaluation of 5HT neuronal activity using radiolabeled tryptophan or 5HI. Problems associated with this technique include the inability to evaluate small tissue sections, unequal distribution of the radiolabeled amino acid, and nonspecific uptake of the label by nontargeted cells.

A primary non-steady-state method of evaluating catecholamine activity includes the measurement of NE or DA depletion following the blockade of the rate limiting enzyme

in catecholamine synthesis, tyrosine hydroxylase. With this method greater neuronal activity is indicated by increased rates of catecholamine depletion following the inhibition of synthesis. Using this method catecholamines can be evaluated in small nuclear regions using sensitive analytical techniques. With the high dosages required to inhibit catecholamine synthesis, some nonspecific effects may occur. Additionally, it cannot be assumed that catecholamine neuron will behave similarly under non-steady-state conditions. Fortunately, studies using different methods often agree.

A more recent method of evaluating monoaminergic neuronal activity includes the measurement of amine and metabolite using amperometric methods. These techniques have not been used extensively in neuroendocrine studies, however.

Norepinephrine

A large body of evidence suggests that central noradrenergic neurons control LH and FSH secretion. Sawyer and colleagues originally showed that a variety of centrally acting adrenergic agents influence the ovulatory release of gonadotropins (Everett et al., 1949; Sawyer, 1952; Barraclough and Sawyer, 1957). Further, these effects were not elicited at the level of the pituitary gland (Weiner and Ganong, 1978). Most investigators today agree that central noradrenergic neurons display both stimulatory and inhibitory influences on the release of gonadotropins.

The activity of noradrenergic neurons appears to change in concert with several LH secretory states. Following ovariectomy or castration the concentration of NE in the hypothalamus increases (Donoso et al., 1967). This suggests an increase in NE metabolism and hence activity using several techniques (Anton-Tay and Wurtman, 1968; Anton-Tay et al., 1970; Coppola, 1971; Kizer et al.,1974; Simpkins et al., 1980). Increased NE neuronal activity has also been noted in the hypothalamus prior to LH hypersecretion on proestrus or following gonadal steroid treatment to ovariectomized rats (Zschaeck and Wurtman, 1973; Loftstrom, 1977; Munaro, 1977; Simpkins et al., 1979). These studies argue for a stimulatory role for noradrenergic systems controlling LH hypersecretion.

Many pharmacological studies support a role for NE in regulating gonadotropin release. Adrenergic agents applied systemically or intraventricularly are presumed to interact with catecholamine receptors or alter monoaminergic neuronal activity. The blockade of NE synthesis with DA-beta-hydroxylase (DBH) inhibitors suppresses pulsatile LH release and LH hypersecretion induced by endogenous steroids prior to ovulation, electrical stimulation of the hypothalamus and gonadal steroid administration (P. Kalra et al, 1972; S. Kalra and McCann, 1973; Drouva and Gallo, 1976a; Gnodde and Schuiling, 1976). This blockade of NE synthesis is overcome by the pretreatment with dihydroxyphenylserine which does

not require DBH for its metabolism to NE, but not by l-DOPA, a precursor in catecholamine synthesis. Additionally, alpha-adrenergic antagonists inhibit LH release in castrated and gonadal steroid treated rats, while neurotoxic agents such as 6-hydroxy-DA can prevent prcestrcus LH release (P. Kalra et al., 1972; Gnodde and Schuiling, 1976; Martinovic and McCann, 1977). Together these data argue fcr a stimulatory role for NE on gonadotropin release.

The administraticn of NE has varying effects on LH release depending cn the mode of administration and experimental paradigm employed. While in vitro evidence suggests that NE can stimulate LHRH secretion from hypothalamic fragments via an alpha-adrenergic mechanism (Ojeda et al., 1982; Miyake, 1983), evidence from in vivo studies indicate that LH secretion may represent a balance of both stimulatory and inhibitory inputs. Intraventricular administration of NE inhibits IH release in ovariectomized rats (Gallo and Drouva, 1979). It is not certain whether this effect is mediated by one particular adrenergic receptor, if it is localized at a site within or cutside the hypothalamus, or if the inhibition occurs through LHRH neurons (Caceres and Taleisnik, 1980, 1982; Leung et al., 1981, 1982).

In ovariectomized rats pretreated with gcnadal steroids the intraventricular administration cf NE stimulates IH release (Krieg and Sawyer, 1976; Vijayan and McCann, 1978;

Gallo and Drouva, 1979). It would appear that in an ovariectomized animal, the inhibitory effects of NE on LH release predominate, while in the presence of gonadal steroids, stimulatory modes of NE on LH release are primarily operative.

Despite many studies, the nature of the coupling of the noradrenergic neuron to the LHRH-containing neuron is a subject of debate. LH secretion is most vigorous when NE is infused in a pulsatile fashion and desensitization ensues with a continuous NE infusion (Gallo, 1982). Yet, a recent study by Estes et al. (1982) suggest that a single dose of the alpha-adrenergic agonist, clonidine, stimulates pulsatile LH release for several hours. This would suggest that noradrenergic neurons have a permissive effect on LHRH pulsation, rather than being the driving force behind each individual pulse. This diffuse functional relationship fits the general lack of a direct anatomical connection between the two systems.

Epinephrin

The recent development of inhibitors of EPI synthesis has allowed the differentiation of effects which could be attributed to either NE or or EPI-containing neurons. On a molar basis EPI is more potent than either NE or DA in eliciting H release when injected into the ventricles of gonadal steroid treated ovariectomized rats (Vijayan and

McCann, 1978). Epinephrine might be important in mediating the positive feedback effects of gonadal steroids in female rats (Adler et al., 1928; S. Kalra 1983). It is not clear however what role EPI may play in regulating LH release in ovariectomized rats or in male rats (Negro-Villar et al., 1979; Crowley et al., 1982; Crowley and Terry, 1981).

Dopamine

A great deal of conflicting evidence exists concerning the role of DA in regulating LH and FSH release in male and female rats. *In vitro* studies suggest that DA stimulates LHRH release from hypothalamic tissue fragments (Schneider and McCann 1969; Rotsztejn et al., 1977). The activity of DA neurons appears to be enhanced after castration in the PCA-AH and prior to gonadal steroid-induced LH hypersecretion in the MBH of female rats (Simpkins et al., 1979, 1980). While this might argue for a stimulatory role for DA neurons on LH release, DA itself does not consistently induce LH secretion when injected into the ventricles (Drouva and Gallo, 1976a; Krieg and Sawyer, 1976; Vijayan and McCann, 1978; Gallo and Drouva, 1979).

Serotonin

The importance of serotonergic neurons in the overall regulation of gonadotropin release is unclear. Intraventricularly administered 5HT stimulates or inhibits

LH release depending on the dose employed (Kamberi et al., 1970; Cramer and Porter, 1973). Studies with the neurotoxin, 5,7-dihydroxytryptamine, suggest that serotonergic neurons stimulate LH release (Wuttke et al., 1978, Van der Kar et al., 1980). While it is uncertain whether 5HT-containing neurons control LH release in ovariectomized rats (Gallo, 1980b), several authors suggest that the stimulating effects of estrogen and P on LH release may be influenced by serotonergic neurons (Iyengar and Rabii, 1983; Walker and Wilson, 1983; Chen et al., 1984). Since many serotonergic drugs also act upon catecholamine neurons or their receptors, more information must accumulate on this subject before a more definitive assessment can be made.

Endogenous Opioid Peptides and the Control of Gonadotropin Secretion

The first experimental evidence that opioids are inhibitory to reproductive function was Barraclough and Sawyer's (1954) observation that morphine blocked ovulation in the rat. It was later verified that this blockade was due to an inhibition of the proestrous gonadotropin surge (Pang et al., 1977). At this time Cicero et al. (1975a, b) demonstrated that chronic treatment with morphine induced changes in the reproductive system of the male rat similar to the effects of narcotic abuse in men, i.e. depressed serum T and diminished secondary sex organ function. These studies indicated that the effects of opiates on

reproductive function could be assessed in a laboratory setting.

Reproductive Pharmacology of Opioids

The antigonadotropic effects of opiate administration are ultimately exerted at the level of the hypothalamus through the inhibition of LHRH release. Many careful studies have eliminated other possible sites of action (Cicero 1980a). Opiate administration has no effect on the metabolism of T, its clearance from the blood, or its fate at target organs. Further the effects of morphine are not exerted at the level of the testes either by effecting T synthesis or the response of the leydig cell to gonadotropins. At the pituitary gland, morphine does not alter the release or synthesis of LH or the response of the gonadotrope to LHRH. In one study however, leucine-enkephalin acutely inhibited the LH secretory response to LHRH (May et al., 1979).

Several lines of evidence suggest that opiates exert their antigonadotropic actions by inhibiting LHRH release. The increased LHRH concentrations in hypophyseal portal plasma which accompanies the proestrous gonadotropin surge are prevented by morphine treatment (Ching, 1983). Also, opiate agonists and antagonists can modulate the release of LHRH from *in vitro* hypothalamic incubations (Rotsztejn et al., 1978; Drouva et al., 1981; Wilkes and Yen, 1981). In one study the LH stimulatory actions of an opiate antagonist

was prevented by treatment with an LHRH antagonist analogue (Blank and Roberts, 1982).

It is likely that the central site for the opiate-LHRH interaction is within the hypothalamus (S. Kalra, 1981). However, some investigators have found LH-inhibitory effects of opioids administered in the amygdala and brainstem (Parvizi and Ellendorf, 1980; Iakoski and Gebhart, 1981 and 1982).

Most studies of opioid effects on reproductive function measure serum LH levels as an index of LHRH output. The acute effects of opioids on LH secretion satisfy the criteria for mediation by an opiate receptor (Cicero, 1980a).

1. In general, all opioids depress serum LH levels. This has been found to be true for both opiate alkaloids and opioid peptides administered systemically as well as opioid peptides administered intraventricularly (Bruni et al., 1977; Cicero, 1980b; Johnson and Rosencrans, 1981; Kinoshita et al., 1981; Kato et al., 1982; Bhanot and Wilkinson, 1983; Leadem and Kalra, 1983; Marko and Romer, 1983;
2. The relative potency of opiates in suppressing LH release parallels their pharmacological efficacy in other preparations such as the displacement of tritiated opiates, the ability to inhibit the contraction of guinea pig illeum, and analgesia (Cicero et al., 1976; Cicero, 1980a).

3. The effects of opiates on LH secretion are reversed by opiate antagonists like naloxone or naltrexone (Pang et al., 1974; Cicero et al., 1976, Bruni et al., 1977., Muraki et al., 1980).
4. Levarotatory isomers of opiate alkaloids are far more potent in inhibiting LH secretion than dextrorotatory isomers (Cicero et al., 1976).

Physiological Inhibition of Gonadotropin Secretion by Opioids

Although the existence of opiate receptors in the brain and hypothalamus and the presence of a pharmacologic response to stimulation of these receptors suggest opioid pathway which effects LH secretion, this does not, per se, verify that EOP normally act to inhibit LH release. If physiologically released EOP do act to inhibit LH secretion, then blockade of opiate receptors with a narcotic antagonist should reverse this inhibition. The ability of naloxone, on its own, to stimulate LH and FSH secretion is the most persuasive and most frequently verified evidence favoring an EOP inhibition of LH secretion (Meites et al., 1979; Cicero, 1980b; S. Kalra et al., 1980; Ferin et al., 1984). If blockade of EOP activity with an opiate receptor antagonist elicits LH secretion, then sequestering EOP with an appropriate antibody might produce the same effect. Antibodies to both beta-endorphin and dynorphin have been found to stimulate LH secretion (Schulz et al., 1981; Forman et al., 1983)

The mechanisms mediating opiate antagonist induced LH secretion are not known. Although naloxone occupies opioid receptors and prevents the ongoing actions of EOP, naloxone only transiently stimulates LH secretion, and like opiate agonists, tolerance develops to its effects on LH secretion (Owens and Cicero, 1981). Another recent study suggests that the stimulation of LH secretion following naloxone injection may reflect prior opiate agonist activity, rather than simple displacement of an opiate agonist from its receptor (Cicero et al., 1983b). In this study a single injection of morphine enhanced the ability of naloxone to elicit LH release for several hours after morphine had been cleared from the brain.

Multiple Opioid Receptors

Several classes of opioid receptors appear to exist. While different classifications are used, there appear to be at least three distinct opioid receptors, termed mu, delta, and kappa. These receptors do not have widely divergent binding affinities, thus distinguishing between the three classes with specific agonists and antagonists has proven difficult Martin, 1981). These three classes may share a common high affinity binding component (Hahn and Pasternak, 1982). It is this high affinity component that appears to mediate the analgesic actions of morphine. In relation to the various opioid alkaloids and peptides, the mu-opioid

receptor appears to mediate the effects of morphine and its congeners. The delta-opioid receptor appears to be more specific for the enkephalins, while the kappa-opioid receptor appears to be more specific for dynorphin (Childers, 1980; Chavkin et al., 1982). Another receptor, called epsilon, has been proposed based on receptor binding studies with beta-endorphin (Law et al., 1979).

Several studies have utilized opiate alkaloids and opioid peptides to discern which receptor subtype may mediate the effects of opioids on LH secretion. Opioid inhibition of LH secretion appears to involve both a mu and kappa-opioid receptor component (Cicero et al., 1983c; Gabriel and Simpkins, 1983; Leadem and Kalra, 1983; Pfieffer et al., 1983). This agrees with studies which show that antibodies to beta-endorphin and dynorphin, but not methionine-enkephalin, stimulate LH secretion (Schulz et al., 1981; Forman et al., 1983).

Opioid-Monoaminergic Interactions

The interaction between opioid and monoamine-containing neurons has been investigated in several systems (Schwartz, 1979; Kuchinsky, 1977). Several recent studies have characterized a possible adrenergic interaction with opioids in the control of gonadotropin secretion. The stimulatory effects of naloxone on LH secretion are prevented by adrenergic antagonists, DBH inhibitors and EPI synthesis

inhibitors (S. Kalra, 1981; S. Kalra and Simpkins, 1981; Van Vugt et al., 1981; S. Kalra and Crowley, 1982; Schulz et al., 1982; Koh et al., 1983; Adler and Crowley, 1984). Additionally, the acute administration of opiate antagonists appears to modulate the activity of catecholamine neurons in the hypothalamus (Adler and Crowley, 1984). It appears that adrenergic neurons may influence LH secretion without an intermediary opioid interaction since the inhibition of LH secretion seen following morphine is reversed by subsequent treatment with clonidine or the intraventricular EPI injection (S. Kalra and Simpkins, 1981; S. Kalra and Gallo, 1983).

In addition to both NE and EPI-containing neurons, EOP have been shown to interact with dopaminergic and serotonergic neurons (Van Loon and De Souza, 1978; Gudelsky and Porter, 1979). There have been reports that the effects of opiates and EOP on LH secretion are influenced by each of these monoaminergic neuronal systems (Rotsztejn et al., 1978; Ieiri et al., 1980b).

Opioid-Gonadal Steroid Interactions

While it is apparent that EOP-containing neurons act to suppress LH release, the function of this inhibitory input is not well understood. Some researchers have presented evidence that EOP may relay the feedback signals of gonadal steroids in the brain. Morphine, like T, can prevent the

post-castration rise in serum LH, while naloxone and T are mutually antagonistic on LH secretion in orchidectomized rats. Similarly estrogens can inhibit naloxone's stimulation of LH secretion in ovariectomized rats (Blank et al., 1979; and 1980; Cicero et al., 1980; Sylvester et al., 1982; Van Vugt et al., 1982)

If opioid neurons do relay the feedback signals of the gonadal steroids on LH secretion, then it can be expected that the pharmacological efficacy of opioid agonists and antagonists will vary under differing reproductive states. In prepubertal female rats, when LH levels are markedly suppressed, naloxone is highly effective in stimulating LH release (Blank et al., 1979). The efficacy of naloxone in stimulating LH secretion appears to vary diurnally and diminish after castration (Blank and Mann, 1981; Cicero et al., 1983). Opioid agonists are more effective in prepubertal rats compared to pubertal rats, and in rats castrated acutely versus rats castrated several weeks (Bhanot and Wilkinson, 1983; Cicero et al., 1982a; Wilkinson and Bhanot, 1983).

An alternate means of evaluating the potential involvement of EOP in mediating gonadal steroid feedback would be to assess changes in ECF concentrations or their release under varying steroid milieus Peptide release would be the most preferable estimate of neuronal activity. To date, only beta-endorphin has been evaluated in the

hypophyseal portal plasma of non-human primates. Cf interest, beta-endorphin levels decline precipitously following ovariectomy and during menstruation (Ferin et al., 1984), This would imply that the activity of hypothalamic beta-endorphin-containing neurons depends on ovarian factors, particularly P.

Tissue levels of EOP show changes which may be relevant to gonadotropin secretion. Both beta-endorphin in the septum and medial preoptic area and methionine-enkephalin in the MBH and POA-AH display circadian variations in tissue concentrations that parallel changes in LHRH levels in the hypothalamus of the male rat (Kumar et al., 1982; S. Kalra et al., 19 1b; Kerdelhue et al., 1973). Orchidectomy does not appear to alter beta-endorphin levels in the hypothalamus but decreased levels of both beta-endorphin and methionine-enkephalin are found in the NIL and anterior pituitary following castration (Lee et al., 1980; Hong et al., 1982; Petraglia et al., 1982; Yoshikawa et al., 1983a,b). Beta-endorphin levels in the hypothalamus appear to increase as male rats approach puberty (Lee et al., 1980).

Rationale

From the literature presented it appears that EOP may function as one of several neurotransmitters that regulate the release of the gonadotropins, LH and FSH. Based on the pharmacological effects of opioid agonists and antagonists during various reproductive states, and the ability of gonadal steroid alterations to modify EOP levels in the brain and pituitary it appears that EOP-containing neurons respond to changes in the steroid milieu. These changes may reflect alterations in EOP neuronal activity which mediate the feedback effects of gonadal steroids on gonadotropin secretion.

This thesis will present a series of pharmacological investigations of opioid neurons in male and female rats. In the male, EOP appear to suppress LH secretion, but the nature of this inhibition is not well understood. These studies will evaluate the feedback effects of the gonadal steroids on LH and FSH secretion in the presence of continuous opiate receptor stimulation with morphine. In the female rat, the extent of EOP inhibition of LH secretion has not been fully assessed. Naloxone was used to evaluate the potential EOP inhibition of LH secretion present during the estrous cycle and following gonadal steroid treatment to ovariectomized rats. Finally, the feedback effects of gonadal steroid treatment on gonadotropin secretion in females was evaluated in the presence of continuous opiate receptor stimulation with morphine.

CHAPTER III
GENERAL MATERIALS AND METHODS

Animals

The laboratory rat was chosen as the experimental animal in these studies. Adult male and female S-D rats were obtained from Charles Rivers Breeding Laboratories in Wilmington, Massachusetts. Animals weighed 180 to 220 grams upon arrival and were allowed several days to adjust to the animal quarters before initiating an experiment. The rat colony was maintained in a light (lights on 0500 h through 1900 h) and temperature (26 ± 1° C.) controlled room with food and water provided *ad libitum*.

Reproductive status of female rats was verified by microscopic examination of vaginal lavages (Ingram, 1956). Rats which displayed two consecutive 4-day estrous cycles were chosen for studies employing gonadal intact female rats. The normal sequence of cell morphology in the vaginal smear consists of lavages containing cornified epithelial cells (estrus), followed by two days of predominately leukocytic smears (diestrus I and diestrus II), which is then followed by a day in which the lavages contain nucleated epithelial cells (proestrus). The cornified epithelium and leukocytic smears are characteristic of a

gonadal steroid milieu dominated by estrogens and progestins, respectively.

Surgical procedures consisted of subcutaneous implantation of drugs or steroids and bilateral gonadectomy performed under light ether anesthesia. Male rats were orchidectomized by exteriorizing the testicles through a midline ventral incision. Female rats were ovariectomized by a bilateral dorsal approach. Animals were monitored for post-surgical wound healing.

Two methods were employed for collecting blood. In most experiments, trunk blood was collected by decapitation. Decapitations were completed within 30 seconds of removal of each rat from its home cage. In studies employing LHRH injection, blood samples were collected by cardiac puncture under light ether anesthesia. All blood samples were collected in a room separate from the animal quarters. Sera was separated from trunk blood by centrifugation (1000 X g) for 15 minutes while jugular and cardiac blood samples were centrifuged in a microcentrifuge for two minutes. All sera were stored at -20° C. for later hormone analysis by radioimmunoassay (RIA).

Dissection of Brain Tissue

Brains were rapidly removed and placed with their dorsal surface on ice. Tissue sections containing the MBH and POA-AH were removed using fine iris scissors. Cuts for the MBH fragment were made at the posterior border of the optic chiasm, then caudally at the level of the mammillary bodies, 2 mm laterally at the hippocampal sulcus, and 2 mm below the ventral surface of the hypothalamus. The boundries of the POA-AH tissue slice were the caudal borders of the olfactory tubercles to the posterior border of the optic chiasm. Additional cuts were placed 2 mm lateral to the midline and approximately 2 mm from the dorsal surface of the POA-AH at the level of the anterior commissure.

Gonadal Steroid Treatment

Gonadal steroids were obtained from Steraloids Inc., Milton, N.J. and administered as subcutaneous implants or as injections. Implants consisted of Silastic tubing (1.57 mm i.d., 3.17 mm o.d.) of lengths ranging from 2.5 mm to 30 mm. Capsules were filled with either crystalline forms of T, 5-alpha-dihydrotestosterone (DHT) or 17-beta-estradiol (E2) or E2 dissolved in sesame seed oil. The implants were sealed at both ends with Silastic adhesive and allowed to dry at room temperature for 24 to 48 hours. Before use, these implants were soaked in phosphate buffered saline for 48 hours. These implants provide sustained blood levels of

gonadal steroids for several weeks. In the male rat these implants reversed post-castration LH hypersecretion at physiologically relevant dosages (P. Kalra and Kalra, 1980). The implantation of crystalline E2 in ovariectomized rats provided sustained E2 levels which immediately reduced LH secretion and stimulates a daily signal for the midafternoon release of LH (Legan et al., 1975).

Two other methods for stimulating midafternoon LH hypersecretion in ovariectomized rats employed the injection of estradiol benzoate (EB) or the sequential administration of EB plus P. Rats which were ovariectomized two weeks previously were injected with 7.5 ug of EB dissolved in 100 microliters of oil at 1000 h. This treatment produced a fall in LH secretion followed by a midafternoon LH surge two days later. If 5 mg of P dissolved in 100 microliters of oil were injected into these rats 48 hours after EB treatment, a more pronounced LH surge with an earlier onset results. Other endocrine changes accompanying this treatment are discussed in Chapter II.

Treatment with Morphine or Naloxone

Morphine dependency was produced by subcutaneous implantation of one pellet containing 75 mg morphine (free base, Merk, St. Louis, MO), 37.5 mg microcrystalline cellulose (Avisil, FMC Corporation, Philadelphia, PA.), 0.56 mg Cab-o-sil (Cabot Corporation, Boston, MA.) and 1.13 mg

magnesium sterate (Fisher Chemical Co. Fair Lawn, N.J.). Two days later, two additional morphine pellets were implanted. The pellets were compounded in this laboratory. This treatment regimen produced morphine dependency as measured by several tests of analgesia and withdrawal (Gibson and Tingstad, 1970; Simpkins et al., 1983b). Control animals received placebo pellets which were formulated with an additional 75 mg. Avisil rather than morphine free base.

Naloxone HCl (Dupont Pharmaceuticals, Garden City, N.J.) was dissolved in normal saline and administered subcutaneously.

Measurement of Catecholamines Indolamines and Metabolites

Concentrations of NE, DA, 5HT, the NE metabolite normetanephrine (NME), the DA metabolites dihydroxyphenyl-acetic acid (DOPAC) and homovanillic acid (HVA) and the 5HT metabolite 5-hydroxy-indolacetic acid (5HIAA) were measured by amperometric methods following their separation by high-pressure liquid chromatography using a modifaction of the procedure described by Michaud et al. (1981). The separation was accomplished using reverse phase chromatography across an IBM LC-18 (15 cm X 4.6 mm, 5 micrometer particle size) with a mobile phase composed of 8% methanol, 0.2 mM octyl sodium sulfate, 0.1 M. NaH_2PO_4 and 0.1 mM EDTA at pH 2.9. The flow rate was varied from 0.5 ml/min for the first 7.5 minutes of separation, 0.7 ml/min

from 7.5 through 23 minutes, 2.0 ml/min from 23 to 39 minutes and 3.0 ml/min thereafter, through 57 minutes. This procedure allowed the elution of catecholamines, indolamines and their metabolites in standards and samples within a one hour period. The times for elution (in order) for NE, NME, DA, DOPAC, 5HIAA and HVA were 5.5, 13, 17.5, 21, 28.5, 33.5 and 36 minutes respectively. The detection of amines, indolamines and their metabolites was accomplished with an electrochemical detector (IBM,model IC 9533) set with the potential difference between the working electrode and a reference AgAgCl2 electrode of 0.9 volts and a current generated at 20 nA/mV.

Tissue sections containing the MBH and POA-AH were dissected from brain tissue as described above. The fragments were homogenized with a tissue sonicator in 0.4 N. perchloric acid containing 1 mg% EDTA at a weight:volume ratio of 1 mg/10 microliters. Average weights of these tissues were 19.0 ± 0.5 mg for MBH and 20.2 ± 0.6 mg for POA-AH.

To each 20 microliter of sample of MBH and POA-AH tissue homogenate was added 2 ng dihydroxybenzo-acetic acid (DHBA) as an internal standard. The concentration of each amine, indolamine, and metabolite was determined by the peak height ratio of the compound to DHBA in relation to a standard curve of peak-height ratios for that particular catecholamine, indolamine, or metabolite. The sensitivity of

this assay was less than 100 pg for NE, DA, 5HT, and their metabolites.

Hormone Radioimmunoassays

Luteinizing Hormone and Follicle Stimulating Hormone

Serum and medium samples were assayed for LH and FSH using the kits provided by the National Institutes of Arthritis, Diabetes and Digestive and Kidney Diseases (NIADDK). The rabbit-derived antisera used were NIADDK-anti-rLH-S-7 for the LH assay and NIADDK-anti-rFSH-S-11 for the FSH assay. Radioiodinations were performed in our laboratory using standard procedures for a chloramine-T iodination with gel filtration to separate free iodine from hormone-bound iodine.

Because these studies were performed over a considerable period of time, LH values were determined in relation to two LH reference standards provided by the NIADDK. The later LH reference preparation, IH-RP-2, was 61 times more potent than the original LH-RP-1. To aid in the comparison of IH values across experiments, all IH values were expressed relative to the original LH-RP-1 standard. The figure or table legends will note when this conversion was made. FSH values were expressed in relation to the reference standard, FSH-RP-2. The intra-assay variation for the LH and FSH assays, determined by the coefficient of variation for 10 replicates of pooled castrate serum which inhibited the

binding of the radiolabeled hormone 40% to 60% was 6.8% and 6.3%, respectively. The inter-assay variation, determined from successive pooled serum in assays performed over a 6 month period was 11% and 8% for LH and FSH, respectively. The sensitivity of these assays, defined as a the amount of standard hormone required to inhibit the binding of the radiolabeled hormone by 20% was 0.90 ng (LH-RP-1) for LH and 0.40 ng for FSH.

Luteinizing Hormone Releasing Hormone

Tissue sections containing the MBH and POA-AH were homogenized in 2 ml of 0.1 N. HCl and supernates were analyzed for LHRH using RIA methods described previously (S. Kalra, 1976). Acid supernates were neutralized with 2 N. NaOH during the assay procedure. Synthetic LHRH obtained from Beckman Co. (Palo Alto, CA.) was used as the reference standard and for iodination. Monoradioiodinated LHRH was employed as described previously (Nett and Adams, 1977). Rabbit antibodies against LHRH were purchased from Miles Laboratories (Elkhart, IN). The minimum sensitivity for this assay was 2 pg per tube and was estimated as the concentration of LHRH which inhibited the total labeled binding by 10%. Concentrations of LHRH were expressed in terms of tissue sample (i.e. ng per MBH or POA-AH).

Testosterone

Serum T levels were analyzed according to procedures described previously (P. Kalra and Kalra, 1982).

Statistical Analysis

For most experiments, analysis of variance with Student Neuman Keuls tests were used to evaluate the significant differences between treatment groups. Where appropriate, Student-t tests were also used. In studies which injected LHRH, paired-t analyses were employed to determine the significant effects of LHRH injection. To further evaluate data in Chapter 4, the regression analysis programs contained in the Statistical Analysis System package offered by the Northeast Regional Data Center were utilized. In all studies a significance level of $p < 0.05$ was required.

CHAPTER IV
THE EFFECTS OF CHRONIC MORPHINE TREATMENT ON TESTOSTERONE NEGATIVE FEEDBACK IN CASTRATED MALE RATS

Introduction

The feedback effects of gonadal steroids on LH secretion are believed to be mediated by the hypothalamus and pituitary (Drouin and Labrie, 1976; S. Kalra and Kalra, 1983). Recent investigations from several laboratories show that morphine or EOP can acutely suppress LH release in intact and gonadectomized rats (Meites et al., 1979; Cicero, 1980; Kinoshita et al., 1981; Ieadem and Kalra, 1983). Interestingly, gonadal steroids have also been found to modify EOP levels in various sites within the hypothalamus and the secretion of beta-endorphin in the hypophyseal portal system (Barden et al., 1981a; Wardlaw et al., 1982; Wehrenberg et al., 1982). Further, acute blockade of central opiate receptors with narcotic antagonists transiently reverses the inhibitory feedback effects of T on LH release (Cicero et al., 1980). Since gonadal steroid treatment and opiate receptor stimulation suppress LH release and EOP-producing neurons are found in the vicinity of LHRH neurons, it is logical to suspect that EOP-containing neurons may either mediate the feedback effects of gonadal steroids or

that they may act through similar hypothalamic mechanisms to decrease LH release (P. Kalra and Kalra, 1980; Watson et al., 1980; Sar and Stumpf, 1975; Shivers et al., 1983b). The following study compares continuous opiate receptor stimulation with morphine to T replacement on LH secretion in castrated rats, and evaluates the feedback sensitivity of T on LH secretion in the presence of chronic morphine treatment.

Experimental

In these studies castrated rats were treated chronically with morphine pellets and tubing containing crystalline T.

Experiment 1

In the first study, rats received either chronic morphine treatment, replacement T therapy (two 15 mm tubes) or control treatment (placebo pellets or empty tubes) which commenced either at the time of or two weeks after castration. Animals were killed by decapitation after 7 days of treatment. Serum was analyzed for LH and T, while the brains were rapidly removed and dissected for analysis of MBH and AH-POA LHRH concentrations.

Experiment 2

In the second study, rats were castrated and two weeks later received either control pellets or implants of morphine, T, or morphine plus T. Testosterone-containing tubes were either 2.5, 5.0, or 10.0 mm in length. Animals were killed by decapitation 4 days later. Serum was analyzed for LH and T, while brains were rapidly removed and tissues dissected for analysis of LHRH concentrations.

Experiment 3

Rats which had been castrated two weeks previously received either control, 5 mm T, or morphine plus 5 mm T implants. After 4 days, rats were killed by decapitation, anterior pituitaries were removed, hemisectioned, and preincubated in control medium (minimal essential medium + 25 mM Hepes, pH 7.2, Gibco Inc., Grand Island, NY) for one hour at 37° C. Fresh control medium or medium containing 1×10^{-7} M. LHRH was then added to the hemipituitary incubation medium and the incubation continued for an additional hour. Medium was stored at -20° C for later analysis of LH levels.

Results

Effects of Time After Castration on the Serum LH and Hypothalamic LHRH Responses to T and morphine

Figure 1 illustrates serum IH concentrations in rats treated with either T or morphine immediately after or two weeks after castration. Serum T levels attained by the two 15 mm implants were 2161 ± 120 pg/ml in rats treated immediately after castration and 2439 ± 129 pg/ml in rats receiving T two weeks after castration. These levels are in the range observed normally in intact male rats (P. Kalra and Kalra, 1980). As evident from Figure 1, when treatment was started at the time of castration (short-term castrate) both morphine and T prevented the post-castration hypersecretion of IH ($p < 0.05$). However, when the initiation of treatment was delayed for two weeks after castration (long-term castrate), unlike T, morphine was no longer effective in suppressing LH secretion.

Levels of LHRH in the MBH of rats treated with T or morphine either at the time of castration or two weeks after castration are shown in Figure 2. Both T and morphine prevented the post-castration decline in MBH LHRH concentrations if the treatments commenced at the time of castration ($p < 0.05$). Two weeks after castration, however, only T was effective in stimulating LHRH accumulation in the MBH.

Levels of LHRH in the AH-POA of castrated rats treated with T or morphine were unaffected by any experimental treatment (data not shown).

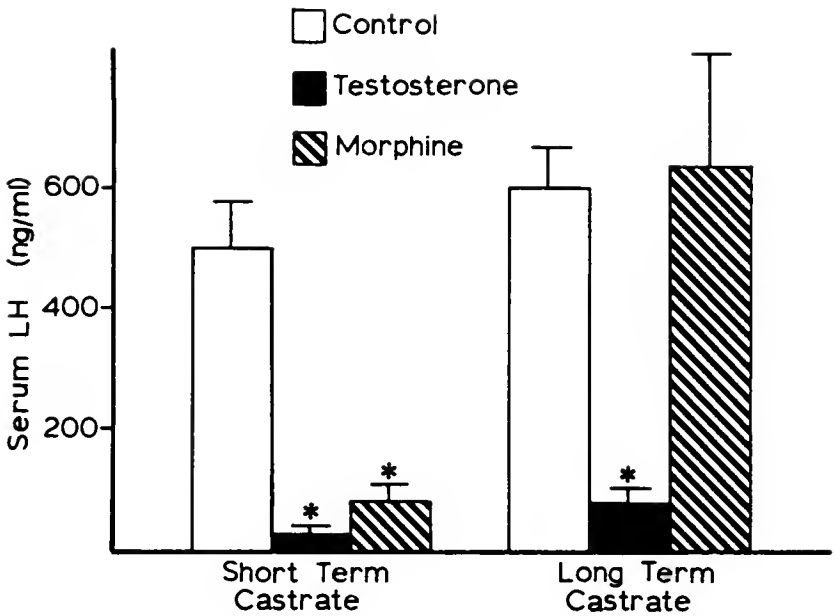

Figure 1: Serum LH concentrations in rats treated with morphine or T at the time of castration or two weeks after castration.

Short-term castrate = treatment started immediately after orchidectomy. Long-term castrate = treatment started two weeks post-castration. * p denotes < 0.05 vs. control. LH concentrations among the control groups were not significantly different and were therefore pooled.

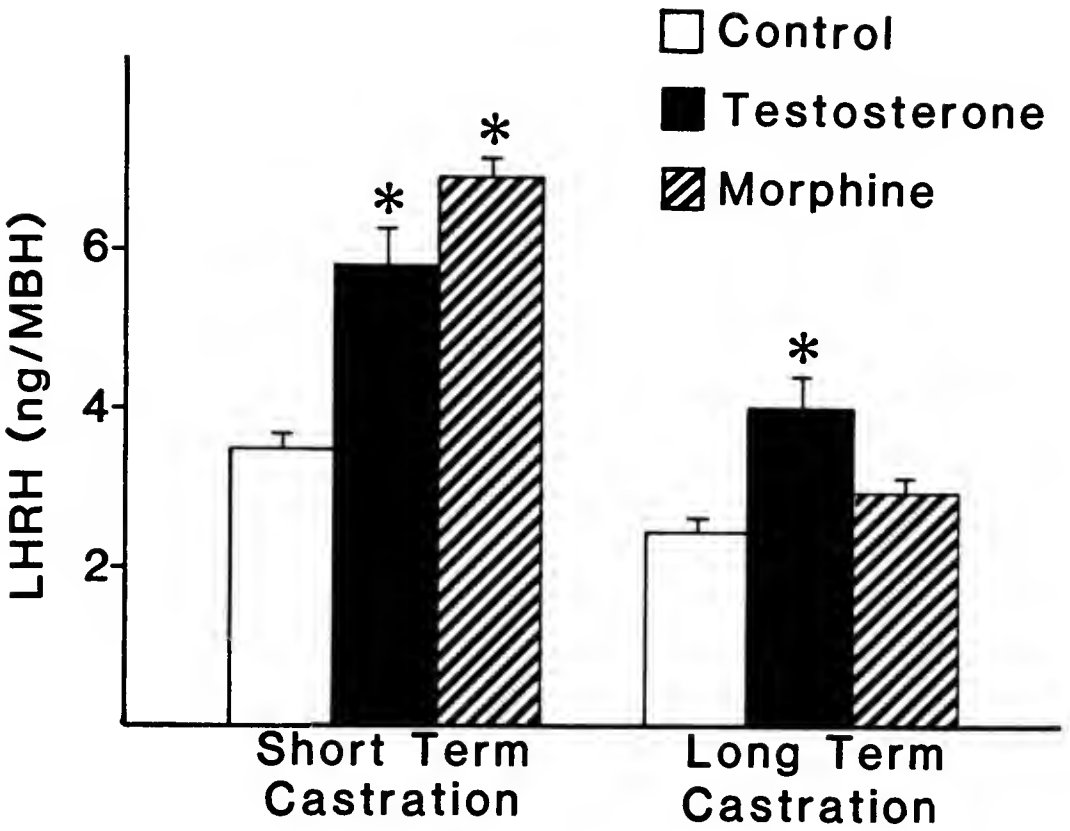

Figure 2: MBH LHRH concentrations in rats treated with morphine or T at the time of castration or two weeks after castration

Short-term castration = treatment started immediately after orchidectomy. Long-term castration = treatment started two weeks post-castration. * p denotes < 0.05 vs. control. MBH LHRH concentrations among the control groups were not significantly different and were therefore pooled.

The Effects of Chronic Morphine Treatment on the LH Secretory and MBH LHRH Responses to Graded Doses of T.

Figure 3 illustrates serum T levels attained from various sized implants in rats castrated for two weeks treated additionally with either morphine or placebo pellets. Low serum levels of T (<100 pg/ml) were detected in morphine or placebo-treated rats receiving only sham implants. There was a progressive increase in serum T levels as the size of the implant increased in both morphine and placebo-treated groups ($p < 0.05$). Chronic morphine treatment did not affect serum T levels attained by any of these implants when compared to placebo-treated groups.

Serum LH concentrations in rats treated with morphine or placebo pellets in combination with graded doses of T are also shown in Figure 3. In placebo-treated rats, low circulating levels of T (355 ± 20 pg/ml, 2.5 mm implant) caused a slight but nonsignificant increase in serum LH. Further increases in serum T levels produced a decrease in serum LH secretion, with significant suppression of LH levels seen at 1.18 ± 0.07 ng/ml of T (10 mm implant). As observed in Experiment 1, morphine treatment alone did not significantly change serum LH levels in castrated male rats. However, it influenced the LH response to T treatment. In rats treated with both morphine and T, the LH response curve to T shifted to the left such that a 50% suppression of

serum LH was observed with the 2.5 mm T implant and nearly complete suppression of serum IH was seen with 5 mm T implants.

To further evaluate the interacticn between morphine and T, the data from this experiment were grouped according to different levels (Figure 4):

1. less than 199 pg/ml, representing ineffective implants, or T levels found in sham-implanted (castrated) animals;
2. between 200 and 499 pg/ml, representing T levels which have no effect on IH or IHRH concentrations;
3. between 500 and 999 pg/ml, representing T levels which stimulate LHRH accumulaticn in the MEH but have little effect on LH secretion; and
4. greater than 1000 pg/ml, representing T levels which consistently suppress LH secretion (P. Kalra and Kalra, 1982).

As evident in both placebo and morphine-treated rats, IH levels declined progressively as a function of serum T levels. However the T-induced reduction of LH concentrations was greatly enhanced in morphine-treated rats ($p < 0.05$). The LH response curve to graded doses of T was shifted to the left in morphine-treated rats with maximal inhibitiсn occuring at 622 ± 44 pg/ml. In some of these morphine-treated rats, near baseline LH levels were fcund with T concentrations as low as 420 pg/ml serum. In contrast, but

in agreement with previous studies (P. Kalra and Kalra, 1982), significant depression of serum LH concentrations was only apparent with T concentrations of greater than 600 pg/ml. Serum T concentrations necessary to elicit a 50% reduction in LH secretion were 300 pg/ml in morphine-treated rats and 960 pg/ml in placebo-treated rats.

The data from Experiment 2 were further subjected to regression analysis using the logarithm of serum LH concentration as a dependent variable, placebo or morphine treatment as an independent variable and the logarithm of serum T concentration as a covariant. The resultant regression model was found to be highly significant ($p < 0.001$) as were the drug treatment (placebo or morphine), T treatment, and the interaction of drug with T ($p < 0.01$, for each).

The effects of simultaneous T plus chronic morphine treatment on LHRH concentrations in the MBH are shown in Figure 5. In placebo-implanted rats, T exposure for 4 days resulted in an accumulation of LHRH in the MBH ($p < 0.05$). As has been noted previously, this accumulation of LHRH in the MBH occured at T levels lower than that required to inhibit LH secretion (5 mm T implants, Figure 5 vs. 10 mm implants, Figure 4; P. Kalra and Kalra, 1982). It can also been seen from Figure 5 that in the presence of morphine, T was unable to cause any significant increases in LHRH stores in the MBH.

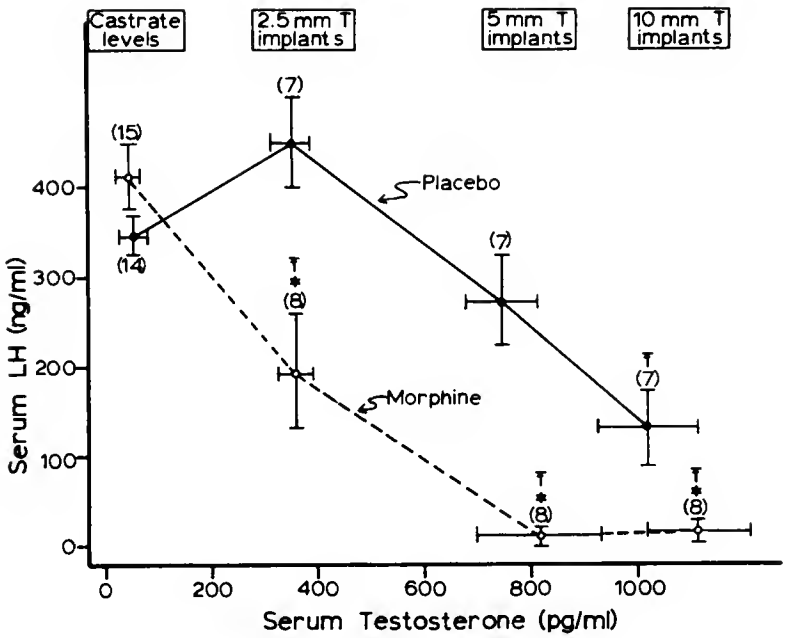

Figure 3: The effects of graded doses of T produced by various sizes of T implants on serum T and LH in morphine-treated and placebo-treated male rats castrated for two weeks

The vertical and horizontal bars represent mean ± standard error for serum LH and T concentrations, respectively. Numbers in parentheses represent the number of rats in each treatment group. * denotes $p < 0.01$ vs. sham-implanted control group; the dagger symbol denotes $p < 0.05$ vs. placebo-implanted group at the same dose of T.

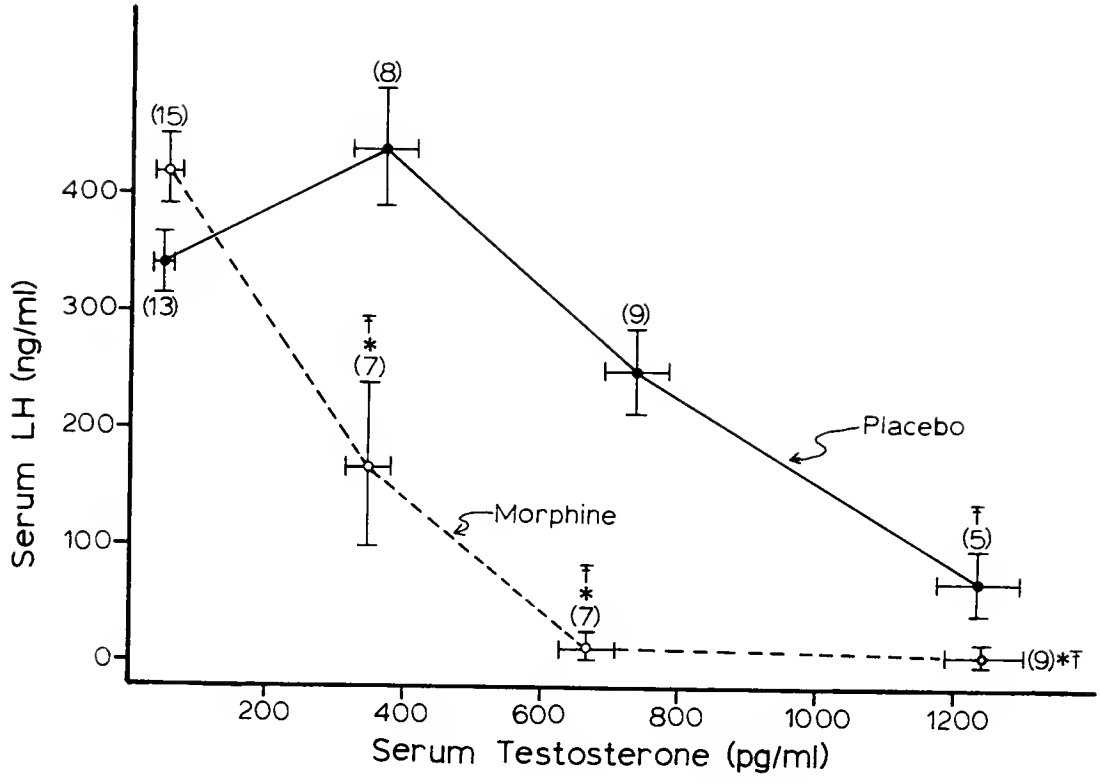

Figure 4: Relationship between LH and T levels in morphine-treated and placebo-treated male rats castrated for two weeks

In the interest of clarity in presenting the relationship between the LH response and T levels, the rats were blocked into 4 groups according to serum T levels; < 199 pg/ml, between 200 and 499 pg/ml, 500 and 999 pg/ml, or > 1000 pg/ml. Number in parentheses represent the number of rats in each T level. The vertical and horizontal error bars represent mean ± standard error for the LH and T concentrations, respectively. * denotes $p < 0.01$ vs. T < 199 pg/ml group; the dagger symbol denotes $p < 0.05$ vs. placebo-implanted group at the same T level.

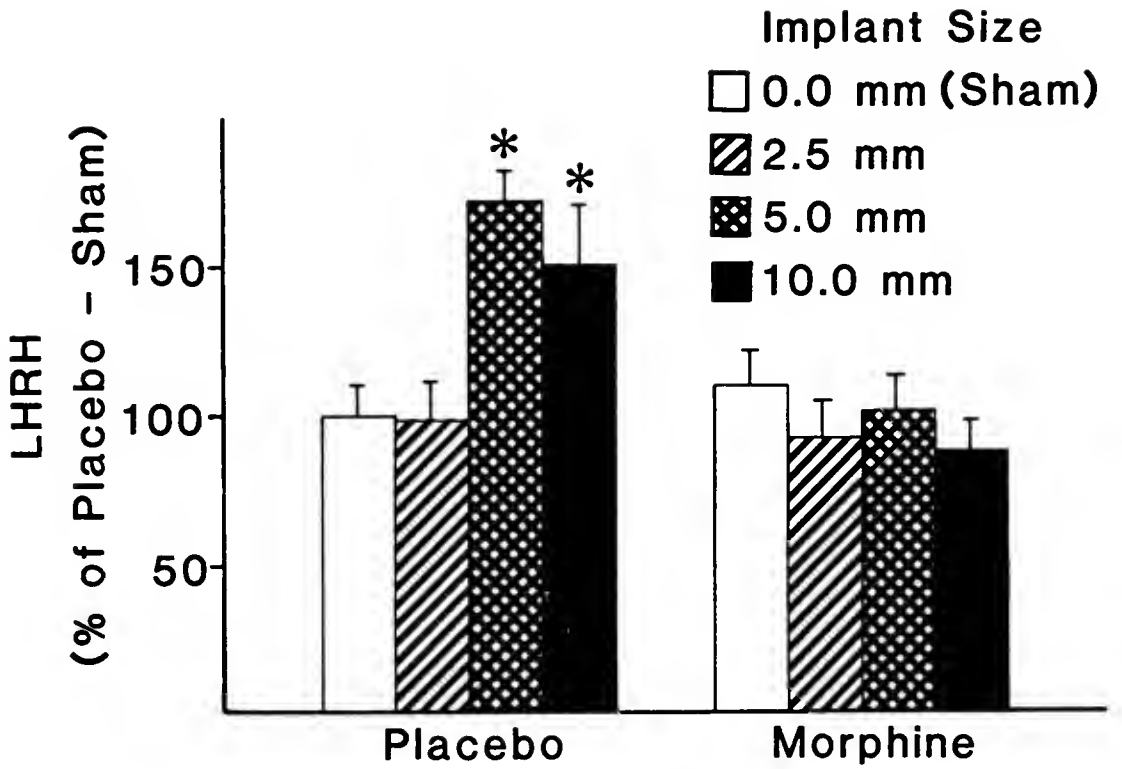

Figure 5: The effects of various sized T implants on MBH LHRH concentrations in morphine-treated and placebo-treated male rats castrated for two weeks.

LHRH concentrations were determined per MBH tissue section and expressed relative to sham-implanted MBH LHRH (control) concentrations. * denotes $p < 0.05$ vs. sham-implanted group.

Effects of T and Morphine Treatment on the Pituitary Responsiveness to LHRH

Testosterone levels achieved by the 5 mm implants were similar in placebo and morphine-treated rats. Once again this T treatment failed to reduce serum LH concentrations in placebo treated rats but together with morphine pellets reduced serum LH concentrations to baseline levels (Table 1, legend, $p < 0.05$)

The effects of the *in vivo* T or morphine plus T treatment on the *in vitro* release of LH from pituitary incubations are also shown in Table 1. Incubation of pituitaries for one hour with 1×10^{-7} M. LHRH significantly increased LH concentrations to levels above that seen in control medium ($p < 0.05$). However, neither T or morphine plus T treatment *in vivo* significantly altered the baseline or the LHRH stimulated levels of LH release.

TABLE 1

Effects of In Vivo Morphine and T Pretreatment on In Vitro LH Release from Hemisectioned Pituitaries (LH release rate: ng/mg pituitary tissue/ hour)

In vitro treatment	In vivo treatment		
	Control	T	Morphine + T
Control	1,583 ± 313	957 ± 51	1,393 ± 166
1 X 10^{-7} M. LHRH	3,930 ± 555	3,682 ± 275	4,588 ± 285
delta-LH	2,346 ± 666	2,612 ± 362	3,194 ± 425

Serum T levels achieved by the 5 mm T implants were 683 ± 89 ng/ml for morphine-implanted and 636 ± 73 pg/ml for placebo-treated rats. Serum LH concentrations were 225 ± 33 ng/ml in castrated (placebo plus sham treated) rats, 227 ± 61 ng/ml in 5 mmT implanted rats, and 16 ± 1 ng/ml in 5 mm T plus morphine treated rats.

Discussion

These studies reveal a putative underlying interaction between opiates and T on LH release. As in the case of T implants, placement of morphine pellets immediately after castration prevented the post-castration rise in serum IH and the decline in IHRH concentrations. This extends the observations of previous studies (Cicero et al., 1980; Van Vugt et al., 1982). However, in contrast to the expected suppression of LH release and stimulation of LHRH concentrations in the MBH after T implantation, LH and its

releasing factor were unaffected by morphine treatment initiated two weeks after castration. Apparantly, opiate receptor stimulation does not mimic the actions of T on the hypothalamic-pituitary-LH axis under all circumstances as has been suggested (Cicero et al., 1980; Van Vugt et al., 1982).

The inability of morphine pellets to suppress LH release in rats which had been castrated for two weeks is surprising in view of the observation that administration of morphine or opioids systemically or ECF intraventricularly promptly suppressed LH release in gonadectomized rats (Cicero et al., 1980; Kinoshita et al., 1981; Leadem and Kalra; 1983). It is quite possible that a similar decrease in LH release may occur soon after morphine pellets are placed in two-week castrated rats. Accordingly then, this LH suppression must be transient because with sustained supply of morphine these rats appear to overcome the inhibition and LH secretion seemingly occured unabated 4 to 7 days later. While this may be a plausible explanation for the absence of LH suppression in long-term castrated rats, it should be noted that acutely orchidectomized rats were unable to override the effects of sustained morphine supply. This and previous reports of a differential LH response to opioid administration which is dependent upon the post-castration interval is intriguing (Cicero et al., 1982a; Bhanot and Wilkinson, 1983). The ability of testosterone to inhibit LH release diminishes

with time after castration (Cicero et al., 1982a). It is possible that similar mechanisms may underlie the loss in effectiveness of both androgens and opioids.

In addition to the findings that in long-term castrated rats morphine is either ineffective or its suppressive effects dissipate rapidly, the action of morphine appears to manifest itself in a different form. This is shown by the observation that low concentrations of T, while failing to exert any impact on LH release on their own, were highly effective in suppressing LH release in morphine-treated rats. The LH response curve to graded doses of T was shifted to the left (Figures 3 and 4) in morphine-treated rats with maximal inhibition occuring at T concentrations of 622 ± 44 pg/ml serum. In some morphine-treated rats, near baseline LH levels were seen with T concentrations as low as 420 pg/ml. Furthermore, it appears that serum T levels needed to achieve a 50% reduction in serum LH levels were three times lower in morphine-treated rats than in control rats.

Evidently morphine treatment concurrently with T rendered rats more responsive towards T feedback action. T has been shown to decrease pituitary responsiveness to LHRH (Drouin and Labrie, 1976; P. Kalra and Kalra, 1980). It is possible that morphine may interact synergistically with T at the level of gonadotropes to suppress pituitary responsiveness to endogenous LHRH stimulation and thereby produce a marked decrease in serum LH levels. However, as shown by the dose

employed in the Experiment 3 and previous studies (Cicero et al., 1977; Wiesner et al., 1984), there was no evidence of modification by morphine of LHRH action at the pituitary level. Thus, one can assume that morphine acts at higher centers, possibly at the preoptico-tuberal pathway where the distribution of androgen concentrating, EOF, and LHRH-producing neurons and opiate receptors overlap (Sarr and Stumpf, 1975; Watson et al., 1980; S. Kalra, 1981; Shivers et al., 1983b).

Precisely how T and chronic morphine interact to inhibit LH secretion is not known. The apparent inability of morphine to reduce LH secretion after two weeks of castration would suggest a T requirement for this effect of morphine. It is possible that after continuous morphine exposure, neuronal systems regulating LHRH release may be more responsive to T, so that extremely low serum T titers can suppress LH release. Considering the ability of chronic morphine to block the accumulation of LHRH in the MBH following exposure, it is possible that chronic morphine suppressed the activity of the LHRH neuron at several steps in the secretory process.

While the LHRH neuron may be a likely site for the interaction between T and morphine, other explanations are possible. Earlier work has suggested that gonadal steroids may modify brain opiate receptors but this possibility has been disputed (Hahn and Fishman, 1979; Cicero et al.,

1983a). The possibility remains, however, that the intracellular processing of the opioid signal requires the presence of androgens. Also other neurotransmitters, such as NE, have been shown to interact with the opiates in effecting LH secretion (S. Kalra and SImpkins, 1981). The potential involvement of moncamines in mediating the interaction between morphine and T will be explored in the following chapter.

CHAPTER V
THE INFLUENCE OF CHRONIC MORPHINE TREATMENT ON THE NEGATIVE FEEDBACK REGULATION OF GONADOTROPIN SECRETION BY GONADAL STEROIDS

Introduction

The negative feedback effects of testicular hormones on gonadotropin secretion appear to be exerted at the level of the hypothalamus and the pituitary (Drouin and Labrie, 1976; Franchimont et al., 1979; S. Kalra and Kalra, 1983). In the male rat, three major gonadal steroids, T, DHT and E2 have been implicated in the feedback regulation of LH secretion, and to a lesser extent, FSH secretion (P. Kalra and Kalra, 1980; D'Agata et al., 1981; Sherins et al., 1982; McCann et al., 1983; Nishihara and Takahashi, 1983). While E2 and DHT can be formed intracellularly from T in many neuroendocrine tissues, all three of these gonadal steroids are present in the circulation in sufficient concentrations to influence LH secretion (Massa et al., 1972; Naftolin et al., 1975; P. Kalra and alra, 1977, 1980, 1981, 1982).

While the neuroendocrine substrates which mediate the feedback effects of steroids on gonadotropin are not known, it is interesting that a close anatomical relationship exists between steroid concentrating, LHRH and EOP-containing neurons (McEwen et al., 1979; Watson et al.,

1980; Shivers et al., 1983b). EOP neuronal systems have been implicated in the central regulation of LH secretion, and a considerable amount of pharmacologic evidence suggests that EOP play a role in modulating the negative feedback effects of gonadal steroids on LH release in the male (Cicero et al., 1980; Van Vugt et al., 1982). As was seen in Chapter IV, chronic opiate receptor stimulation with morphine, while ineffective in inhibiting LH release on its own, enhanced by 3-fold the negative feedback effects of T. The present study extends these observations by comparing the effects on LH and FSH secretion of T, DHT and E2 in male rats treated chronically with morphine.

Experimental

Chronic Morphine and Gonadal Steroid Treatments

Groups of placebo or morphine-treated rats were simultaneously exposed to either sham implants or one of the three gonadal steroids at various dosages. All treatments lasted for 4 days, after which animals were sacrificed by decapitation between 1100 h and 1300 h. Serum from trunk blood was stored at -20° C. for subsequent analysis of LH and FSH by RIA. The steroid treatments were:

1. 5 mm tubes packed with crystalline T;
2. 7.5 mm tubes packed with crystalline DHT or DHT which had been diluted with cholesterol on a weight:weight ratio of 1:1 or 1:3; and

3. E2 dissolved into sesame seed oil at a concentration of 300 micrograms/ml and filled into 7.5 mm tubes or E2 diluted in oil to concentrations of 150 or 75 micrograms/ml and then placed into tubes of 5.0 mm in length.

Evaluation of Pituitary Responsiveness to LHRH

Based on the results of the first series of experiments, groups of placebo or chronic morphine treated rats were simultaneously exposed to either sham, 5 mm T, 7.5 mm DHT or 7.5 mm E2 (300 micrograms/ml) implants. After 4 days of the above treatments, rats then received a single injection of LHRH (100 ng/100 g B.W., s.c.). Blood samples were obtained by cardiac puncture under light ether anesthesia, prior to, and 30 minutes after LHRH injection. This dose of LHRH was based on earlier work (Lu et al., 1980). Serum was separated by centrifugation and stored at -20° C. for analysis of LH by RIA.

Results

The effects of simultaneous morphine plus T administration are shown in Figure 6. While 5 mm T implants alone reduced serum LH concentrations by greater than 40%, this effect was not significant. Chronic morphine treatment alone did not affect serum LH levels in castrated rats. However, as was noted in Chapter IV, the combination of 5 mm

T plus morphine treatment reduced serum LH concentrations by 90% to levels seen in intact male rats ($p < 0.05$; P. Kalra and Kalra, 1977b).

The consequences of chronic morphine exposure on serum LH and FSH levels in castrated rats concurrently exposed to various dosages of E2 are shown in Figures 7 and 8, respectively. Chronic morphine did not alter serum LH concentrations in animals receiving sham implants. Similarly, E2 treatment alone failed to significantly reduce LH levels. In contrast, the combination of morphine treatment plus 5 mm E2 (150 micrograms/ml) or 7.5 mm E2 (300 micrograms/ml oil) reduced serum LH concentrations ($p < 0.05$). The highest E2 dosage (7.5 mm at 300 micrograms/ml) alone produced a non-significant 25% reduction in LH concentrations, while the combination of morphine plus the same E2 dosage caused a greater than 75% reduction in serum LH levels $p < 0.05$).

A significant effect of chronic morphine on the response of FSH to E2 was observed. Although E2 alone did not reduce serum FSH concentrations at any of the doses evaluated, the combination of 7.5 mm E2 (300 micrograms/ml) plus morphine significantly reduced FSH levels by 30% relative to sham-implanted rats and 18% relative to placebo-treated rats at the same E2 dosage ($p < 0.05$).

The effects of chronic morphine administration on the response of LH and FSH to various dosages of DHT in

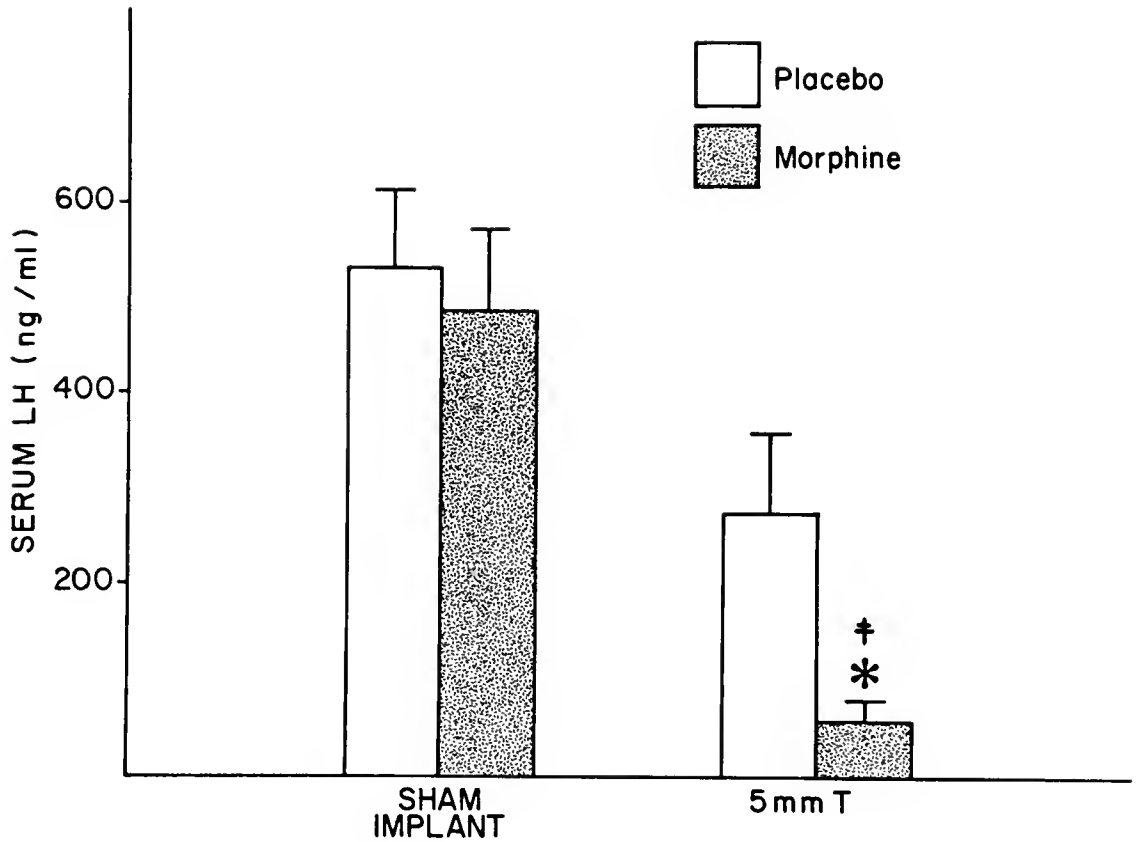

Figure 6: The effects of simultaneous morphine and 5 mm T implants on LH secretion in rats which had been orchidectomized two weeks previously

* denotes $p < 0.05$ compared to sham-implanted rats; the dagger symbol denotes $p < 0.05$ when compared to placebo-implanted rats.

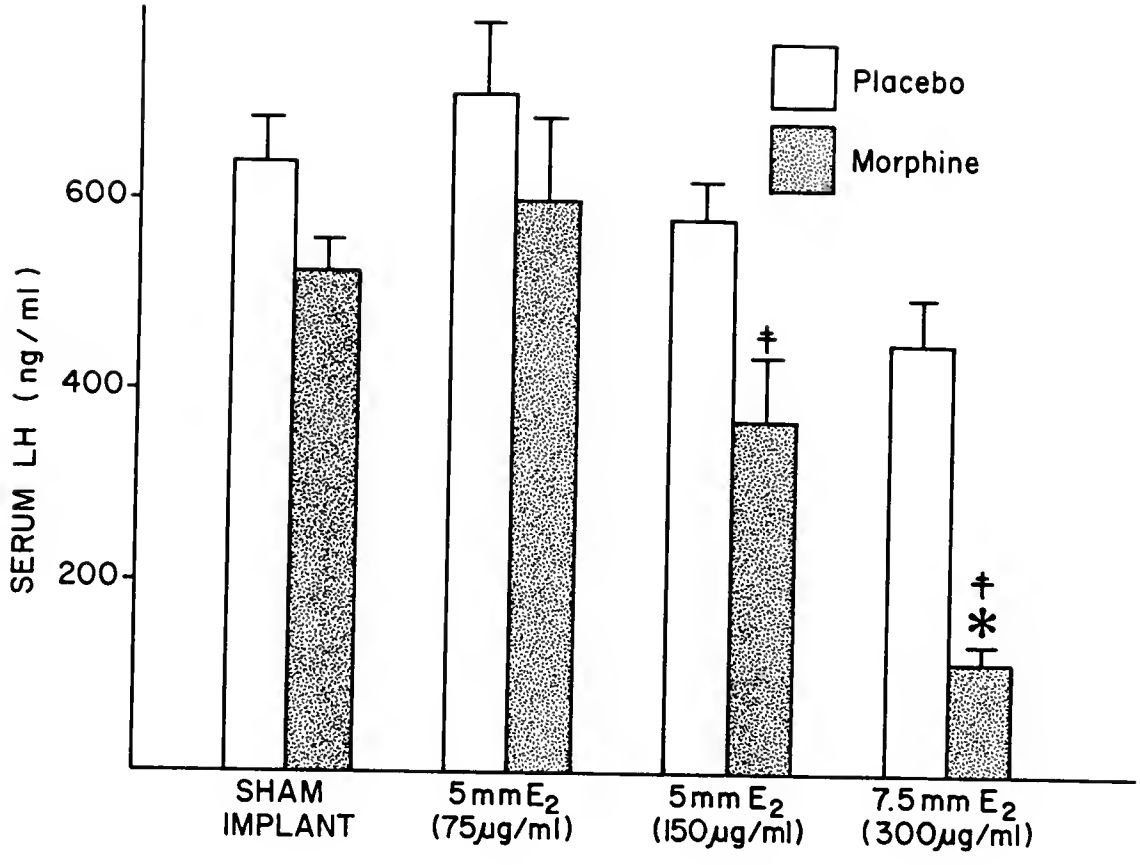

Figure 7: The effects of simultaneous treatment with morphine and various doses of E2 on LH secretion in rats which had been orchidectomized two weeks previously.

E2 was dissolved in oil at the concentrations in parenthesis and filled into tubes of the lengths noted in the figure. LH was determined using the LH-RP-2 reference standard and expressed relative to LH-RP-1 (LH-RP-1 = 61 X LH-RP-2). * denotes $p < 0.05$ vs. sham-implanted rats; the dagger symbol denotes $p < 0.05$ vs. placebo-implanted rats at the same E2 dose.

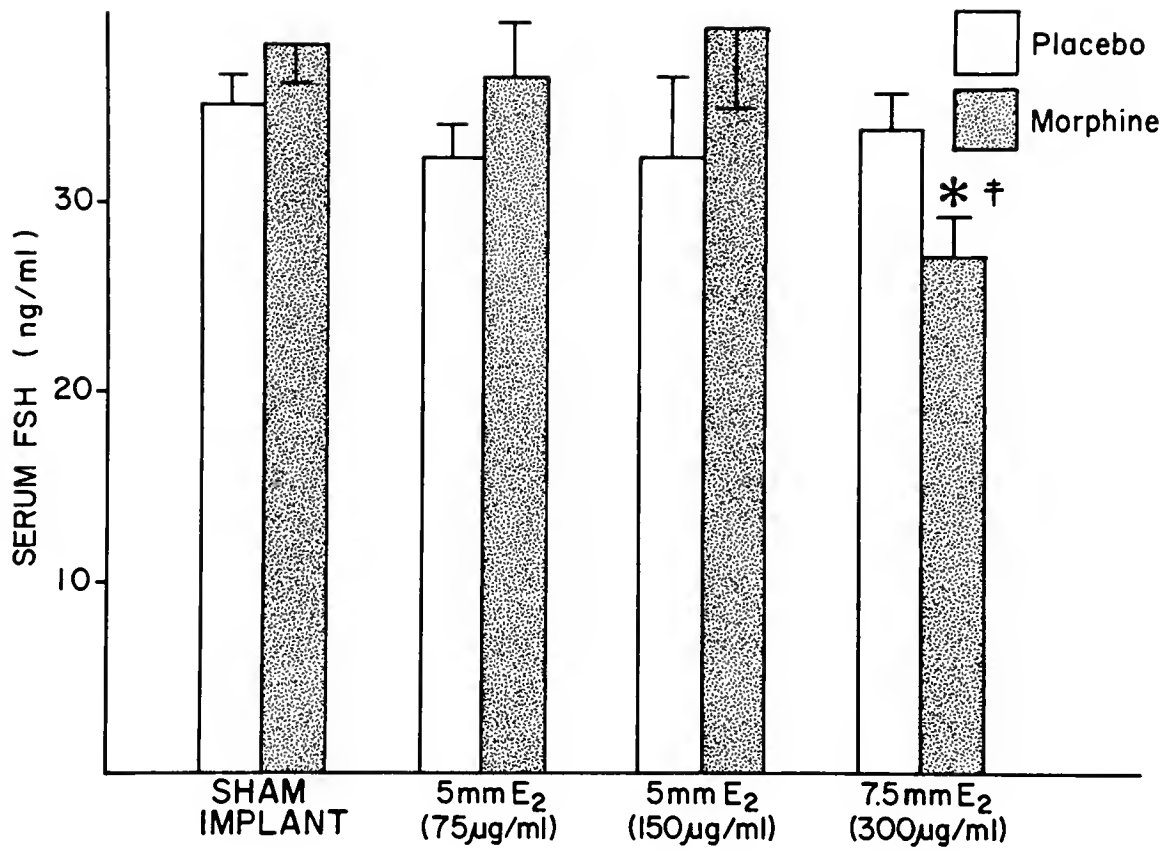

Figure 8: The effects of simultaneous treatment with morphine and various doses of E2 on FSH secretion in rats which had been orchidectomized two weeks previously

E2 was dissolved into oil and filled into tubes of lengths as noted in the figure. * denotes $p < 0.05$ vs. sham-implanted rats; the dagger symbol denotes $p < 0.05$ vs. placebo-implanted rats at the same E2 dose.

castrated rats are shown in Figures 9 and 10, respectively. As previously shown in Figures 6 and 7, chronic morphine treatment was without effect on serum LH levels in sham-implanted rats. The implantation of DHT alone (7.5 mm DHT crystals) significantly reduced serum LH concentrations by 63%, while the combination of chronic morphine plus DHT (at 1:1) or 7.5 mm DHT reduced LH levels 39% and 83%. The combination of morphine plus DHT was not significantly more effective in inhibiting LH levels than DHT alone at any DHT dosage, however. Chronic morphine and DHT had variable effects on FSH levels. Morphine treatment caused a slight elevation (16%) in serum FSH in sham-implanted rats, in contrast to similarly exposed animals shown in Figure 8. Treatment with DHT alone was ineffective in inhibiting FSH at any dose used; however, 7.5 mm DHT (at 1:1) caused a slight increase in serum FSH levels (9%, $p < 0.05$). Morphine treatment with 7.5 mm DHT reduced serum FSH levels 43%. This reduction in serum FSH concentrations was significant relative to sham-implanted rats and to placebo-impanted rats at the same DHT dosage.

The effects of combinations of chronic morphine plus steroid treatments on the *in vivo* LH secretory response to LHRH injection are shown in Table 2. Prior to LHRH administration, the various morphine plus steroid treatments produced similar effects on LH levels as was seen in Figures 6, 7 and 9. Chronic morphine treatment was without effect

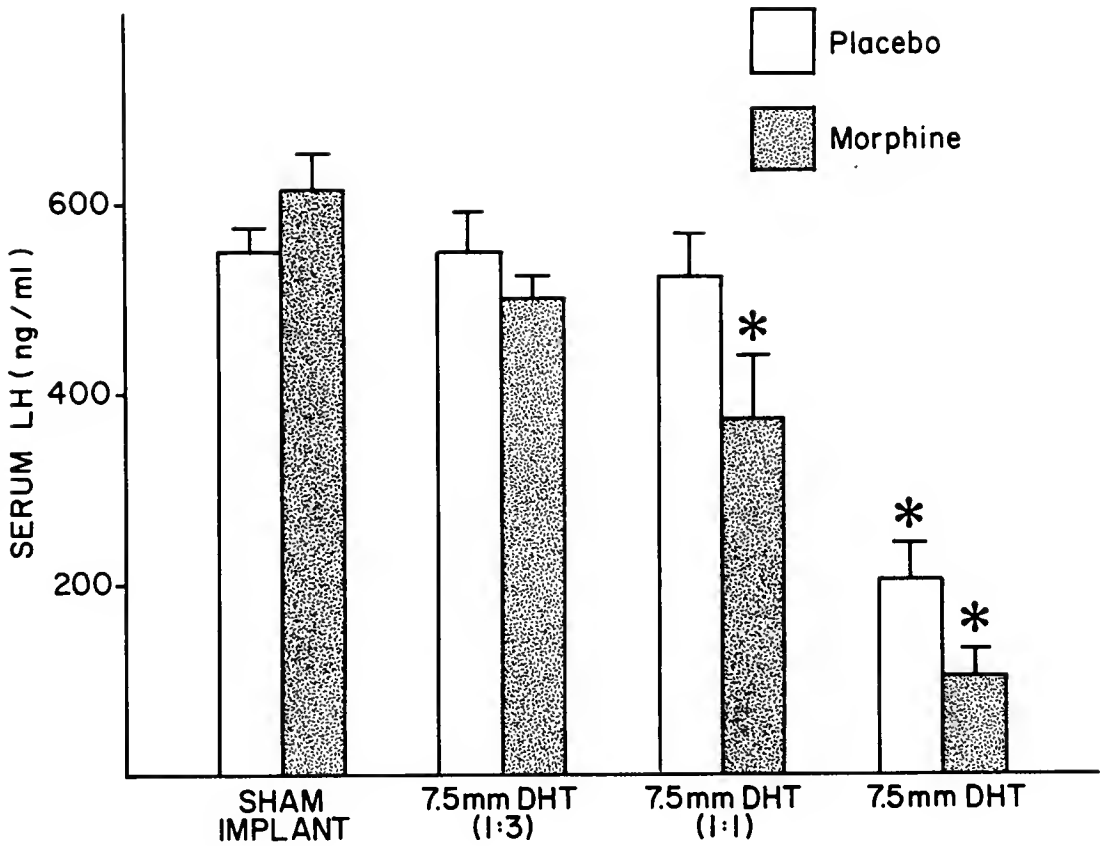

Figure 9: The effects of simultaneous treatment with morphine and various doses of DHT on LH secretion in rats which had been orchidectomized two weeks previously

Crystalline DHT or DHT which had been diluted on a weight:weight basis with cholesterol was packed into tubes 7.5 mm in lengths as noted in the figure. Serum LH was determined using the LH-RP-2 reference standard and expressed relative to LH-RP-1. (LH-RP-1 = 61 X LH-RP-2). * denotes $p < 0.05$ vs. sham-implanted rats; the dagger symbol denotes $p < 0.05$ vs. placebo-implanted rats at the same DHT dose.

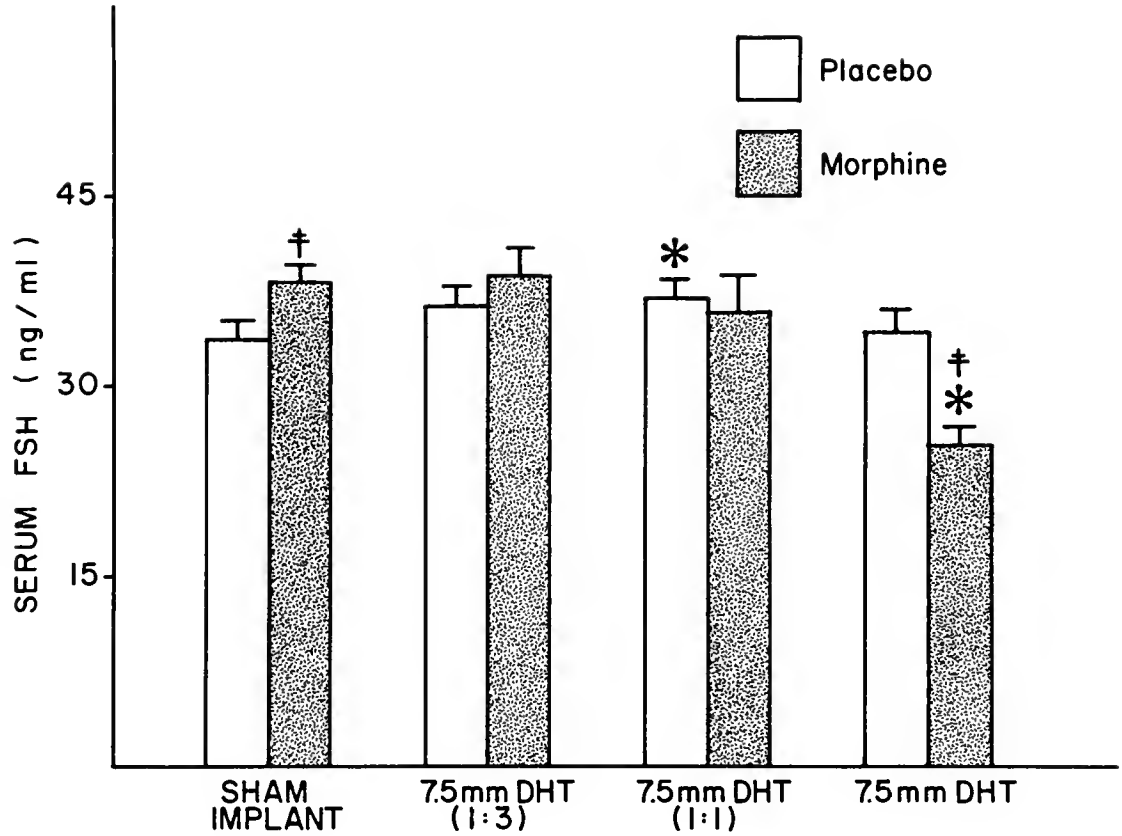

Figure 10: The effects of simultaneous treatment with morphine and various doses of DHT on FSH secretion in rats which had been castrated two weeks previously.

Crystalline DHT or DHT which had been diluted on a weight:weight ratio with cholesterol was packed into tubes 7.5 mm in length as noted in the figure. * denotes $p < 0.05$ vs. sham-implanted rats; the dagger symbol denotes $p < 0.05$ vs. placebo-implanted rats at the same DHT dose.

on serum LH levels in sham-implanted rats prior tc LHRH injection. When compared to sham-implanted rats, 5 mm T was without effect, while 7.5 mm E2 (300 micrograms/ml oil) and 7.5 mm DHT reduced serum LH levels by 57% and 69%, respectively ($p < 0.05$). As expected, in these stercid-treated rats, opiate receptor stimulation with morphine further reduced LH concentrations prior to LHRH injection. However, in 7.5 mm DHT implanted rats this additional reduction was not significant.

LHRH injection stimulated LH release in all 8 treatment groups (p 0.01). When compared tc placebo plus sham implanted (castrate) controls, T exposure did not alter the pituitary response to the decapeptide. Additicnally, although the combination of morphine plus T reduced LH concentrations before LHRH injection, this combination did not alter the pituitary response to LHRH. In rats treated with E2 alone, pituitary responsiveness to LHRH was increased significantly. Despite a reduction in LH concentrations prior to IHRH injection in morphine plus E2-implanted rats, the LH secretcry response tc LHRH was further enhanced. Finally, DHT alone diminished the responsiveness of the pituitary tc LHRH and this reductien in sensitivity was not modified by morphine exposure.

TABLE 2

The Effects of LHRH (100 ng/100 g B.W., s.c.) on serum LH Levels in Castrated Rats Treated Chronically with Morphine and/or Gonadal Stercids.

	Pre-LHRH	Post-LHRH	delta-LHRH
	LH (ng/ml serum)		
Placebo - Implanted Group:			
Sham Implant	832 ± 109	2049 ± 122	1213 ± 67
5 mm T	622 ± 176	1653 ± 298	1140 ± 323
7.5 mm E2	355 ± 79[1]	2110 ± 256	1732 ± 195[1]
7.5 mm DHT	253 ± 113[1]	1091 ± 197[1]	835 ± 146[1]
Morphine - Implanted Group:			
Sham Implant	683 ± 54	2135 ± 176	1451 ± 183
5 mm T	162 ± 75[1,2]	1421 ± 134	1250 ± 91
7.5 mm E2	122 ± 18[1,2]	3281 ± 433[1,2]	3147 ± 439[1,2]
7.5 mm DHT	89 ± 18[1]	965 ± 143[1]	854 ± 146[1]

E2 was dissolved in oil at a concentration of 300 micrograms/ml and filled into tubes 7.5 mm in length. LH was determined using the LH-RP-2 reference standard and expressed relative to LH-RP-1 (LH-RP-1 = 61 X LH-RP-2). [1] denotes $p < 0.05$ vs. sham-implanted rats. [2] denotes $p < 0.05$ vs. placebo-implanted rats within the same steroid treatment group.

Discussion

The results of the present study confirm the findings presented in Chapter IV that, while ineffective on its own, chronic morphine treatment greatly enhances the feedback inhibition by T of LH secretion in rats which had been castrated two weeks previously. This observation is extended by the finding that chronic morphine also enhances the negative feedback effects of E2 and DHT on LH and FSH secretion. Since stimulation of opiate receptors by EOP should similarly affect the gonadotropin secretory mechanism, these data argue for a major role of EOP-containing neurons in regulating the sensitivity of the hypothalamus to circulating gonadal steroids.

When initiated at the time of castration, chronic morphine can block the subsequent rise in serum LH concentrations (Chapter IV; Cicero et al., 1980; Van Vugt et al., 1982). However, chronic opiate receptor stimulation with morphine is unable to suppress gonadotropin levels in rats castrated two weeks previously (Figures 1, 3, 4, and 6 - 10). This castration-induced loss in the ability of opioids to inhibit LH secretion cannot be satisfactorily explained by alterations in hypothalamic EOP levels (Lee et al., 1980) or the number of opioid binding sites in the brain (Cicero et al., 1983a). Rather, this influence of castration on the response of LH to EOP or morphine would

appear to result from a synergistic action between gonadal steroids and opioids (Chapters IV and V; Cicero et al., 1982, Ehanot and Wilkinson, 1983). Since morphine enhances the negative feedback sensitivity of gonadal steroids on gonadotropins, the effectiveness of morphine in suppressing LH soon after castration likely results from the persistence of gonadal steroids in the circulation, or the gradual loss of some steroid-dependent process.

The effects of morphine on gonadotropin secretion appear to be mediated centrally, since the present results (Tables 1 and 2) and *in vivo* and *in vitro* studies indicate that morphine and EOP do not act at the pituitary to inhibit LH secretion (Cicero, 1980b; Weisner et al., 1984). In contrast, it is well documented that gonadal steroids can act locally at the gonadotropes to alter their sensitivity to LHRH (Drouin and Labrie, 1976). At the dosages administered, variable effects of T, DHT and E2 on the LH secretory response to LHRH were noted. These dosages were chosen because they had little effect on LH secretion when administered alone, but decreased LH release when administered with morphine. The 5 mm T implant, which provides low physiological circulating T levels of around 700 pg/ml in castrated rats (Chapter IV; Damassa et al., 1976; P. Kalra et al., 1982), did not alter pituitary responsiveness to LHRH. T has been shown to decrease the response of the pituitary to LHRH, but at much higher doses

than were employed in this experiment (Verjans et al., 1974; P. Kalra and Kalra., 1980). Thus the interaction between morphine and T in suppressing LH release is not due to their individual or combined action on the pituitary.

Many studies suggest that DHT is more potent on a molar basis than T in reducing LH secretion (Verjans et al., 1974; Martini et al., 1979). This is in part due to a direct action on gonadotropes (Verjans et al., 1974; Verjans and Eik-Nes, 1976, 1977). The less dramatic interaction between DHT and morphine in reducing LH secretion may be due to the overriding effects of DHT on LHRH sensitivity. Nevertheless, the combined effects of DHT on pituitary responsiveness to LHRH and its interaction with opioids in suppressing LH release, likely contribute to the ability of DHT to inhibit LH release at concentrations lower than the physiological range (< 140 pg/ml; Coyotupa et al., 1974; Saksena et al., 1978; Saksena and Lau, 1979; P. Kalra and Kalra, 1980).

E2 treatment increased the LH secretory response to LHRH, as has been demonstrated previously (Verjans and Eik-Nes, 1976; P. Kalra and Kalra; 1980). Since at the dosages used, E2 alone did not modify serum LH levels, the alterations in pituitary responsiveness are likely associated with reduced LHRH release from the hypothalamus. In combination with morphine, E2 substantially reduced serum LH concentrations despite a marked increase in the ability of the pituitary to

respond to LHRH. Thus, under simultaneous treatment with this steroid and morphine, release of LHRH from the hypothalamus must be decreased to an extent greater than would be anticipated on the basis of serum LH values alone. The doses of E2 administered have been shown to produce serum E2 levels in the range of 15 to 30 pg/ml. Since E2 levels in male rats have been variously reported to range from 10 to 50 pg/ml (Ewing et al., 1977; Saksena et al., 1978; Saksena and Lau 1979; Keel and Abney, 1980; P. Kalra and Kalra 1981; Nishihara and Takahashi, 1983), alterations in EOP neuronal activity within the hypothalamus could interact with circulating E2 to physiologically modify LHRH release from the MBH.

The effects of LHRH, gonadal steroids and opioids on FSH secretion are, in general, less pronounced than their effects on LH release (Mahesh et al., 1975; Sherins et al., 1982; McCann et al., 1983). Similarly, the present data show that the gonadal steroid-opioid interaction is less effective in suppressing FSH relative to LH release. Factors other than LHRH and gonadal steroids are operative in the regulation of FSH secretion (Steinberger and Steinberger, 1976; McCann et al., 1983; Mizunuma et al., 1983, Lumpkin et al., 1984). This may have contributed to the present failure to document a synergistic interaction between gonadal steroids and morphine in suppressing FSH secretion. This relative refractoriness of the FSH secretory mechanism

to chronic morphine with or without concurrent gonadal steroid treatment, is consistent with previous experiments which have used acute exposure to opiates (Eruni et al., 1977; Delitalia et al., 1981; Grossman et al., 1981; Hemming et al., 1982).).

All three of the gonadal steroids evaluated could be involved in the regulation of LH secretion (P. Kalra and Kalra, 1980, 1981, 1982; Nishihara and Takahashi, 1983). E2 appeared to interact most potently with morphine to inhibit LH release. In contrast, DHT did not show a substantial interaction with morphine and appeared to exert its primary effect directly on the pituitary gland. This existance of a hypothalamic feedback modulator for E2, in preference to DHT, argues in favor of the aromatization of T to E2 or a direct effect of circulating E2 on the hypothalamus as the major steroid feedback mechanism regulating LHRH release in the male rat. Additionally, a direct pituitary effect of DHT to suppress LHRH responsiveness could further diminish LH secretion. Thus, T, E2, and DHT may act in concert at separate sites to achieve basal serum LH levels seen in male rats.

The major finding of the present chapter is that chronic morphine treatment may simulate the effects of activation of opioid receptors by EOP and hence indicate a mechanism by which EOP can interact with gonadal steroids to regulate LH release. The low, physiological levels achieved by these

implants (P. Kalra and Kalra, 1980, 1981, 1982), indicates that such an interaction may be an important mechanism by which the sensitivity of the hypothalamus to steroids is regulated. It is of interest that, in the rat, steroid hormones can alter beta-endorphin concentrations (Wardlaw et al., 1982), and in the monkey, the release of EOP is clearly steroid dependent (Ferin et al., 1984). By modifying activity in one or more EOP neuronal systems, steroid hormones could alter the sensitivity of the hypothalamus to their own negative feedback effects.

CHAPTER VI
THE EFFECTS OF CHRONIC MORPHINE AND TESTOSTERONE TREATMENT ON CATECHOLAMINE AND INDOLAMINE METABOLISM AND GONADOTROPIN SECRETION IN MALE RATS

Introduction

The release of the gonadotropins, LH and FSH, appears to be under the primary stimulatory influence of LHRH and possibly a distinct FSH releasing factor (S. Kalra and Kalra, 1983; McCann et al., 1983). In the male, the feedback actions of gonadal steroids modulate the release of LHRH from the hypothalamus, as well as the response of the pituitary to LHRH (Drouin and Labrie, 1976; S. Kalra and Kalra, 1983). The elucidation of the neurochemical substrates responsible for LHRH output from the hypothalamus and the negative feedback effects of gonadal steroids has been a subject of investigation for several decades. Two neuronal systems which appear to be intimately involved in the release of LHRH are the catecholamines (P. Kalra et al., 1972) and the EOP-containing neurons (Meites et al., 1979; Cicero, 1980b; S. Kalra et al., 1980). Both of these neuronal systems have been implicated in the regulation of gonadal steroid feedback in the male (Cicero et al., 1980; Van Vugt et al., 1982). As was seen in the previous two chapters, chronic morphine treatment, while unable to

inhibit LH secretion on its own, enhances the ability of T to inhibit LH release in rats castrated for two weeks. Several studies indicate that the opiates interact with catecholamines and indolamines to effect LH secretion (Rotsztejn et al., 1978; Ieiri et al., 1980a; S. Kalra and Simpkins, 1981). In this chapter the metabolism of DA, NE and 5HT was evaluated in castrated male rats treated with morphine and T.

Experimental

Male rats which had been castrated two weeks previously were treated with chronic morphine and T as described in the General Materials and Methods section of this dissertation. The treatment groups were: sham plus placebo treatment (castrated rats), 5 mm T, chronic M, or morphine plus 5 mm T treatments. For comparison, an additional group of gonadal intact rats and castrated rats receiving replacement levels of T (30 mm T implants, approximately 2 ng/ml, Chapter IV) were given sham plus placebo treatments. All treatments lasted for 4 days, after which blood was collected by decapitation for LH and FSH analysis by RIA and brain tissue containing the MBH and POA-AH tissue fragments were analyzed for catecholamines, indolamines and their metabolites as described in General Materials and Methods.

Results

Serum LH and FSH

The effects of control, 5 mm T, morphine or 5 mm T plus morphine treatment on LH secretion are presented in Figure 11. Analysis of variance revealed a significant effect of both morphine and 5 mm T treatment ($p < 0.05$) as well as a highly significant effect of morphine plus T treatment ($p < 0.01$). Morphine treatment alone was without effect when compared to castrated rats (sham plus placebo treatment), while 5 mm T treatment resulted in a slight, nonsignificant lowering of LH levels. The combination of morphine plus 5 mm T resulted in a 65% reduction in serum LH levels when compared to castrated rats ($p < 0.05$). Although treatment of castrated rats with the morphine and 5 mm T implants did not return LH levels to those found in gonadal intact animals, treatment with 30 mm T implants alone resulted in LH levels lower than those found in intact male rats (figure legend, $p < 0.05$).

Serum from the same samples were assayed for FSH, the results of which are depicted in Figure 12. Both morphine and 5 mm T, when administered alone were ineffective in altering serum FSH concentrations in comparison to castrated male rats (sham plus placebo treatment). The combination of 5 mm T with morphine reduced FSH levels 39% compared to castrated rats ($p < 0.05$). While FSH levels in castrated rats receiving 5 mm T plus morphine implants or 30 mm T

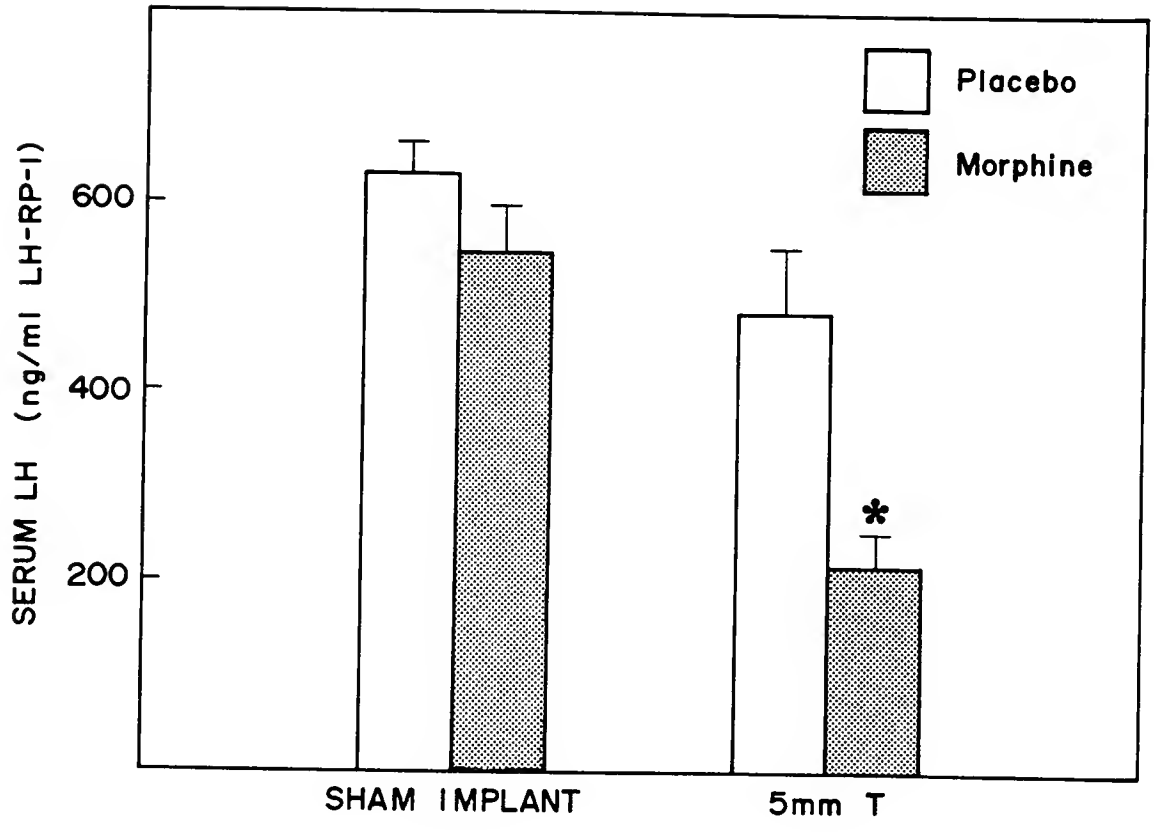

Figure 11: Interaction between morphine and T on LH secretion in rats which were orchidectomized two weeks previously.

LH concentrations in castrated rats receiving 5 mm T plus morphine treatment were significantly greater than levels seen in castrated rats receiving 30 mm T implants (58 ± 2.6 ng/ml) or in gonadal intact rats (78 ± 4.6 ng/ml). LH values were determined using the LH-RP-2 reference preparation and were expressed relative to the LH-RP-1 reference standard (IH-RP-1 = 61 X IH-RP-2). * denotes $p < 0.05$ vs. castrated rats receiving sham implants.

treatment were similar, both groups were significantly elevated when compared to gonadal intact rats (figure legend).

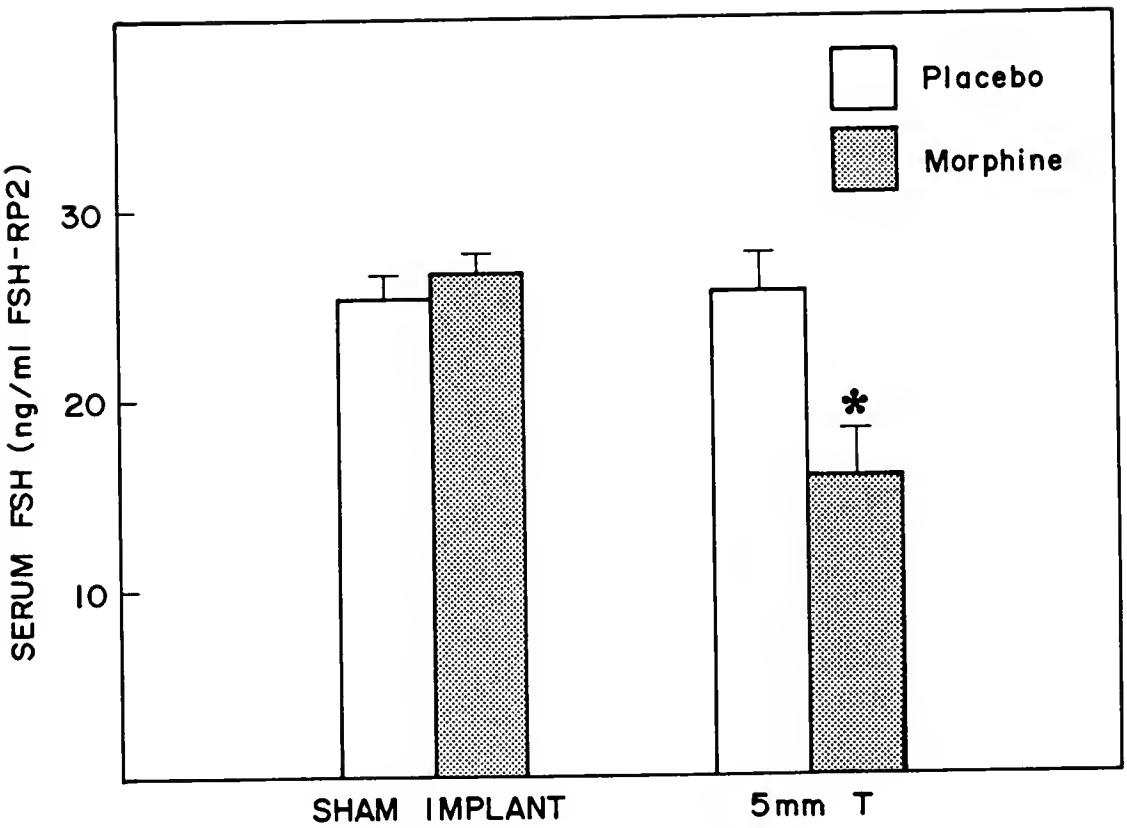

Figure 12: Interaction between morphine and T on FSH secretion in rats which had been castrated two weeks previously.

Serum FSH concentrations in rats receiving 5 mm T plus morphine implants were similar to FSH levels in 30 mm T implanted rats (15.7 ± 0.7 ng/ml), but both groups were significantly greater than FSH levels in gonadal intact rats (8.4 ± 0.4 ng/ml). * denotes $p < 0.05$ vs. castrated rats receiving sham implants.

NE and NME

In the MBH of castrated rats, NE concentrations were elevated when compared to gonadal intact male rats or castrated rats receiving 30 mm T implants (Figure 13, $p < 0.05$). Treatment of castrated rats with 5 mm T or morphine alone significantly reduced NE concentrations. The combination of morphine plus T, while reducing NE levels compared to castrated rats, did not produce any additional reduction in NE levels than T or morphine alone. There were no significant differences in NME levels in the MBH between any of the treatment groups.

There were no differences in NE or NME concentrations in the POA-AH among any of the treatment groups.

DA and DOPAC

In the MBH of castrated rats, DA concentrations were elevated in comparison to intact male rats or castrated rats treated with 30 mm T implants (Figure 14, $p < 0.05$). Castrated male rats treated with 5 mm T alone displayed reduced DA concentrations in the MBH ($p < 0.05$). MBH DA levels in rats receiving morphine alone or morphine with 5 mm T treatment were similar to DA concentrations in rats given 5 mm T implants alone. Morphine or morphine plus T treatment groups were not significantly lower than castrated rats given control implants.

Levels of DOPAC in the MBH were also increased following castration when compared to intact rats or castrated rats given 30 mm T treatment ($p < 0.05$). While both 5 mm T and morphine alone reduced DOPAC levels relative to castrated rats, this effect was only significant in rats given the combination of morphine and 5 mm T.

Levels of DA and DOPAC were similarly affected in the POA-AH. Two weeks after castration DA levels were increased relative to both intact rats and castrated rats given 30 mm T treatment, while DOPAC levels were increased when compared to intact controls ($p < 0.05$). The treatment of castrated rats with 5 mm T implants did not reduce DA or DOPAC levels significantly, but morphine given alone or in combination with 5 mm T did reduce DA levels in comparison to castrated rats.

5HT 5HIAA and HVA

There were no significant effects of any treatment on the levels of 5HT, 5HIAA, or HVA in either the MBH or POA-AH (Table 3).

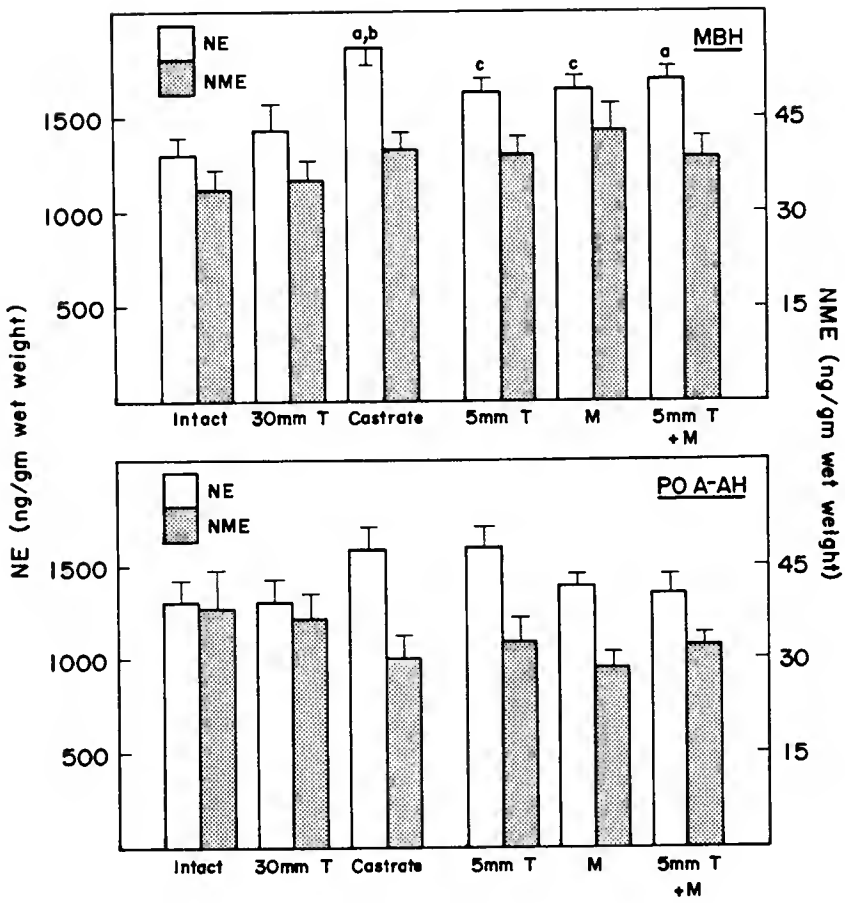

Figure 13: Concentrations of NE and NME in the MBH and POA-AH of intact rats and orchidectomized rats given combinations of morphine and T.

The left-hand scale represents NE concentrations, while the right-hand scale represents NME concentrations. a - denotes $p < 0.05$ vs. intact male rats; b - denotes $p < 0.05$ vs. castrated rats given 30 mm T implants; c - denotes $p < 0.05$ vs. castrated rats given control treatments.

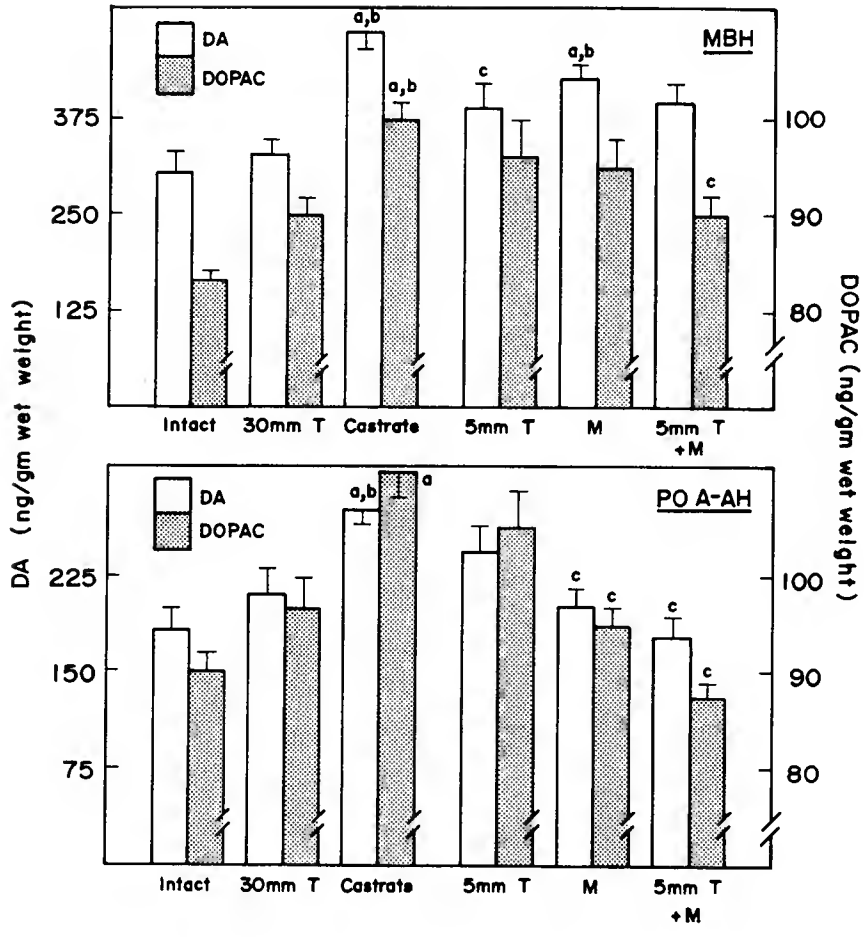

Figure 14: Concentrations of DA and DOPAC in the MBH and POA-AH of intact rats and orchidectomized rats given combinations of morphine and T

The left-hand scale represents DA concentrations, while the right-hand scale, which contains a break, depicts DOPAC concentrations. a - denotes $p < 0.05$ vs. gonadal intact male rats; b - denotes $p < 0.05$ vs; castrated rats given 30 mm T implants. c - denotes $p < 0.05$ vs. castrated rats given control implants.

TABLE 3

The Effects of Gonadal Steroids and Morphine Treatment on 5HT 5HIAA and HVA Concentrations in the MBH and POA.

MBH Region:

	5HT	5HIAA	HVA
	(ng/gm wet tissue wt)		
Intact	254 ± 2	282 ± 3	23 ± 4
30 mm T	256 ± 10	274 ± 4	28 ± 3
Castrate	252 ± 6	275 ± 9	26 ± 3
5 mm T	255 ± 4	289 ± 2	31 ± 3
M.	260 ± 7	288 ± 6	35 ± 3
M. + 5mm T	260 ± 5	285 ± 5	31 ± 4

POA-AH Region:

Intact	252 ± 5	309 ± 9	44 ± 5
30 mm T	265 ± 5	309 ± 9	43 ± 3
Castrate	271 ± 7	315 ± 7	47 ± 2
5 mm T	267 ± 5	314 ± 7	43 ± 3
M.	268 ± 6	319 ± 5	38 ± 6
M. + 5 mm T	261 ± 5	309 ± 2	39 ± 8

Castrated rats were given either 30 mm T implants, 5 mm T implants, chronic morphine alone (M.) or morphine plus 5 mm T implants (i.e. M. + 5 mm T). Gonadal intact and castrated rats were given sham plus placebo implants.

Discussion

The results of the present study demonstrate the existence of a potent interaction between morphine and T in the inhibitory feedback regulation of gonadotropins. While it appears that both morphine and T can alter catecholamine metabolism, an interaction between the androgen and the opiate on catecholamine metabolism, similar to that demonstrated for LH and FSH, was not found. On its own, the 5 mm T implant does not alter serum LH levels (Figures 1, 3, 4 and 6; P. Kalra and Kalra, 1982). These implants provide circulating T levels of approximately 650 pg/ml and are effective in restoring LHRH concentrations in the MBH and median eminence to levels seen in intact male rats (Figures 3 and 5; P. Kalra and Kalra, 1982; P. Kalra et al., 1984). Continuous morphine exposure does not alter LH concentrations in rats which were castrated two weeks previously, in agreement with the castration-induced attenuation in the inhibitory influence of opiates on LH secretion seen in the present work and in previous studies (Figures 1 and 2; Cicero et al., 1982a; Bhanot and Wilkinson, 1983). As noted before, while each are ineffective on their own, the combination of morphine plus 5 mm T potently inhibits LH release (Figures 3, 6 and 11). This effect is apparently mediated centrally since the effects of morphine cannot be attributed to changes in the

response of the pituitary to LHRH or to morphine-induced changes in T metabolism (Figure 3; Tables 1 and 2; Cicero, 1980b; Wiesner et al., 1984).

The present study also demonstrates that the interaction between morphine and T in inhibiting LH release is generalized to include FSH release. This extends the findings of the previous chapter that morphine in combination with E2 or DHT can reduce FSH levels more effectively than treatment with the steroid alone (Figures 8 and 10). The combination of 5 mm T and M, each ineffective on their own, reduced FSH to levels seen in castrated rats given 30 mm T implants. Morphine and EOP acutely inhibit LH secretion (Meites et al., 1979; Cicero, 1980b; Leadem and Kalra, 1983), but are less effective in reducing FSH release (Bruni et al., 1977; Ieiri et al., 1980a; Delitalia et al., 1981; Grossman et al., 1981; Hemmings et al., 1982). Since FSH has a longer half-life in plasma compared to LH (Cobel et al., 1969), the present chronic morphine treatment regimen may have contributed to the differences in this study. The inability of 30 mm T implants to suppress FSH levels in castrated rats to levels seen in gonadal intact animals indicates that physiclcgical replacement with T (approximately 2 ng/ml serum) is not sufficient in returning FSH to concentrations to basal levels, and supports the existence of other gonadal factors which regulate FSH release in addition to T (Steinberger and Steinberger, 1976;

Franchimont et al., 1979). It is not certain whether treatment with morphine plus higher doses of T than emplcyed here (eg. 15 mm or 30 mm T) would further reduce FSH concentrations.

Castration produced an increase in NE ccncentrations and T replacement produced a dose-related decrease in NE levels. Since a gonadal steroid reversible increase in MBH NE levels after gonadectomy has been noted in some (Donoso et al., 1967) but not all studies (Simpkins et al., 1980), it is not certain that this change in NE concentration reflects an increase in NE turnover often noted in this regicn after castraticn (Anton-Tay and Wurtman, 1968; Simpkins et al., 1980). In the present study, NME concentrations did nct change in concert with NE, suggesting that the observed change in NE concentraticns did nct reflect NE release. In this respect treatment with a 5 mm T implant or morphine decreased MBH NE concentration without altering NME levels, and the combination of 5 mm T with morphine did not further changes NE or NME levels. Thus, it appears likely that the interaction between gonadal steroids and morphine in inhibiting LH release is not reflected by changes in NE metabolism. This contrasts with experiments emplcying noradrenergic acting agents which suggest that the effects of opiates on LH release are mediated by NE-containing neurons (S. Kalra and Simpkins, 1981).

Interestingly, dopaminergic neurons in both the MBE and POA-AH appeared to be stimulated by castration since levels of both DA and DOPAC were increased two week after castration. Further, T produced a dose-related decrease in both DA and its major acid metabolite. The effects of T on the incertohypothalamic DA system appears to be inhibitory (Simpkins et al., 1980, 1983a). However the reported effects of T replacement on MBH DA metabolism appears to depend on the specific nuclear regions sampled and the methods used to estimate DA turnover (Fuxe et al., 1978; Simpkins et al., 1983a). Chronic morphine treatment reduced activity in incertohypothalamic DA neurons but was ineffective in altering DA metabolism in the MBH. Also, the combination of morphine with T did not further reduce DA or its metabolite more than the two administered alone. Thus, the interaction between morphine and T in inhibiting LH and FSH secretion is not clearly reflected by a change in DA metabolism or activity. Using other means of evaluating DA neuronal systems it is still uncertain whether DA mediates opioid effects on LH release (Rotsztejn et al., 1978; Sirinathsinhji and Martini, 1984).

Of the three neurotransmitters evaluated, 5HT was the least responsive to any experimental treatment. Levels of 5HT and its acid metabolite, 5HIAA, were unaffected by castration, T treatment, or morphine alone and in combination with T. Some evidence exists for a role of

serotonergic neurons in the control of LH release and in mediating opioid effects on LH release (Ieiri et al., 1980a). The present investigation argues against changes in 5HT metabolism as a stimulus for the interaction between morphine and gonadal steroids in inhibiting gonadotropin release. Similarly, the DA metabolite, HVA, was unaffected by the experimental treatments in this study. In view of the marked changes in DA and DOPAC after several treatments noted above, it would appear that HVA does not serve as a sensitive index of DA neuronal activity or metabolism.

Collectively these studies argue against a role for DA, NE, or 5HT neuronal systems in mediating the potent interaction between morphine and gonadal steroids in inhibitng LH and FSH secretion. Presumably, then, this steroid interaction is manifested at the level of some other neuron, such as the LHRH neuron or the ECP containing neuron.

CHAPTER VII
MODULATION OF ENDOGENOUS OPIOID INFLUENCE ON LUTEINIZING HORMONE SECRETION BY ESTROGEN AND PROGESTERONE

Introduction

Considerable evidence indicates that EOP containing neurons may exert an inhibitory influence on LHRH neurons in the hypothalamus (Meites et al., 1979; Cicero, 1980b; S. Kalra et al., 1980). Among the most persuasive evidence suggesting a role for EOP in the inhibition of LH secretion is the observation that the blockade of opiate receptors with naloxone stimulates LH secretion. This is presumably due to preventing an ongoing endogenous opioid inhibition of LH secretion. Several studies suggest that the influence of EOP on LH secretion may vary considerably during various phases of reproduction and that the magnitude of LH stimulation after naloxone injection may accurately reflect the existing suppressive influence of EOP. While effective in increasing serum LH levels in acyclic prepubertal females, naloxone failed to stimulate LH release in adult cycling rats (Blank et al., 1979). Such an observation suggests that EOP are not operative after sexual maturation in the female rat, in contrast to the adult male rat (Cicero, 1980b). In addition, estrogen treatment inhibited

stimulation of LH release by naloxone in immature female and adult ovariectomized rats (Blank et al., 1979; Blank et al., 1980). More recently, estrogen and P treatment have been shown to alter EOP levels in the hypothalamus and hypophysial portal plasma, and there is evidence for fluctuation during the estrous cycle in some opioid peptide levels in the hypothalamus, pituitary and systemic circulation (Dupont et al., 1980; Barden et al., 1981a; Ishizuka et al., 1982; Knuth et al., 1984; Lim and Funder, 1984). These findings indicate that ovarian steroids may modulate the influence of EOP on IHRH neurons, and this may constitute a mode of feedback action by gonadal steroids. In the present study the potential EOP involvement in the regulation of LH secretion during the rat estrous cycle and in ovariectomized rats pretreated with gonadal steroids was carefully evaluated. In these experiments the LH response after naloxone treatment served as an indicator of potential EOP influence on LH secretion.

Experimental

These studies employed normally cycling adult female rats or rats which had been ovariectomized for two weeks before being treated with EB or EB plus P.

Experiment 1

This experiment used cycling female rats and compared the LH response after naloxone treatment during the rising phase of estrogen secretion (2000 h on diestrus II), at the time of maximal elevations in serum estrogen levels (0800 h and 1200 h on proestrus), and before (1400 h on proestrus), during (1600 h and 1800 h on proestrus), and after (0800 h and 1400 h on estrus) the preovulatory LH surge. Additionally, the LH response was evaluated during the period of elevated P secretion on diestrus I (0800 h; S. Kalra and Kalra, 1974a). Rats were administered naloxone (2 mg/kg in saline, s.c.) or saline (control) at these designated times and killed by decapitation exactly 15 min later. Trunk blood was collected, and serum was stored frozen at -20° C. for subsequent determination of LH concentrations by RIA. The dose of naloxone employed in this and subsequent experiments (2 mg/kg) is comparable to that which has been shown to elicit near maximal LH release in male rats and has been used previously to evaluate naloxone-induced LH responses in adult female rats (Bruni et al., 1977; Blank et al., 1979; Blank et al., 1980; Cicero, 1980b; S. Kalra and Simpkins, 1981).

Experiment 2

In this experiment, the influence of EB alone or EB and P on the naloxone-induced LH response was analyzed. Two weeks after ovariectomy, rats received EB (7.5 micrograms, in oil, s.c., at 1000 h). Two days later, they were divided into two groups. One group received no further steroid treatment (EB group), while the second group received P (5 mg in oil/rat, s.c.) at 1000 h (EBP group). The EB-treated rats were further subdivided into five groups, which then received either saline (control) or naloxone (2 mg/kg, s.c.) on day 2 at 1000, 1200, 1400, 1600, or 2000 h. Similarly, EBP-treated rats were further subdivided into five groups, which then received naloxone or saline on day 2 at 1200, 1400, 1600, 1800, or 2000 h. All animals were killed by decapitation 15 min later, and serum obtained from trunk blood was analyzed for LH levels by RIA.

Experiment 3

In this study the influence of P administration on the naloxone-induced LH response on proestrus was analyzed. Proestrous rats received an injection of P (5 mg/rat, s.c.) at 0900 h. The naloxone-induced LH response in these rats was examined at 1400 h and 1600 h by administering naloxone (2 mg/kg, s.c.) or saline. A control group received oil vehicle instead of P at 0900 h, followed by naloxone or saline at 1400 h. Rats were decapitated 15 min later, and

serum from trunk blood was stored frozen for later analysis of LH by RIA.

Results

Effects of Naloxone Administration During the Estrous Cycle on LH Release

Naloxone administration elicited significant increases in serum LH levels at every stage of the estrous cycle studied (Figure 15 . On diestrus II, LH levels increased by 211% from control values after naloxone injection at 2000 h ($p < 0.05$). A similar magnitude of response was evident the following day at 0800 h and 1200 h and just before the preovulatory LH release at 1400 h on proestrus. In addition, naloxone was effective in eliciting significant LH release during the period of the preovulatory LH surge. Serum LH levels in controls at 1600 h and 1800 h were 1615 ± 386 and 1952 ± 381 ng/ml, respectively, whereas in naloxone-treated rats, they were 4497 ± 767 and 3105 ± 460 ng/ml, respectively, at those times ($p < 0.05$). After the LH surge on proestrus, naloxone injection also elicited a 5-fold increase in LH at 0800 h and a 10-fold increase at 1400 h on estrus ($p < 0.05$). A group of rats (not shown in Figure 15) was injected with naloxone or saline on diestrus day 1 at 0800 h. Serum LH levels in saline-treated rats were 61.22 ± 10.87 ng/ml, whereas they were significantly elevated to 214.79 ± 27.68 ng/ml in naloxone-injected rats ($p < 0.05$).

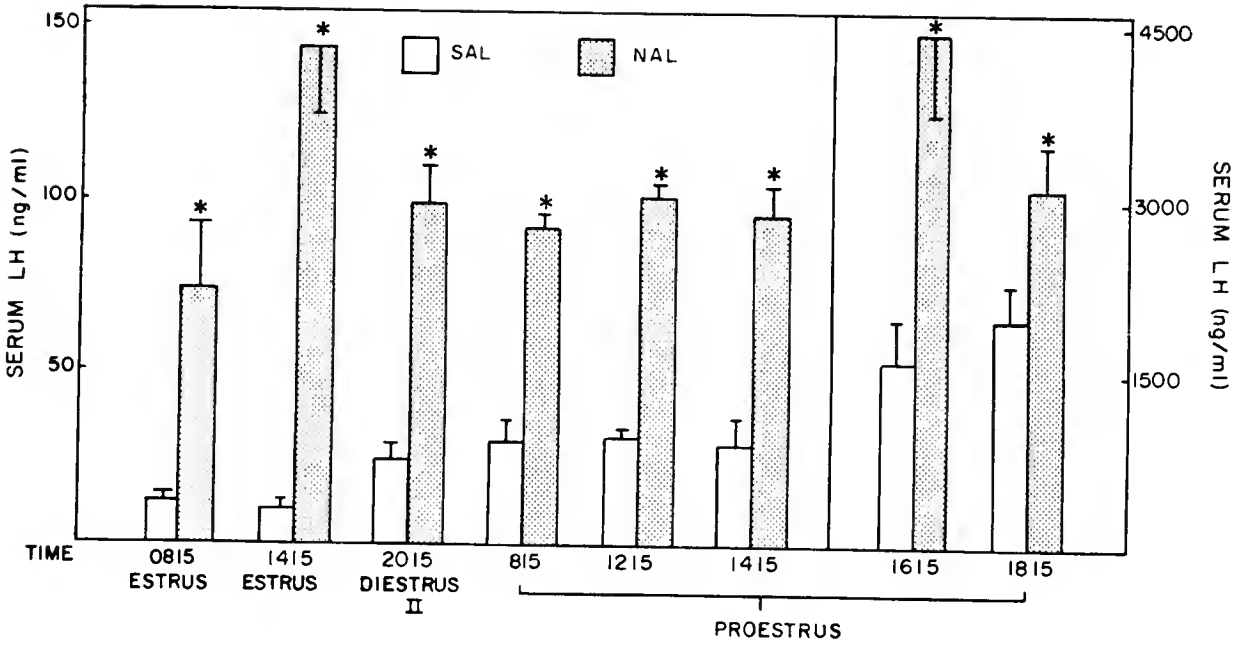

Figure 15: The effects of naloxone (2 mg/kg) on serum LH levels at various times during the estrous cycle.

The time of sacrifice, noted on the abscissa, was 15 minutes after naloxone (NAL) or saline (SAL) injection. Note the scale change for LH concentrations in groups injected during the preovulatory surge (1615 h and 1815 h on proestrus). * denotes $p < 0.05$ vs. saline control at the same injection time.

Effects of Naloxone on LH Release in Steroid - Pretreated Ovariectomized Rats

In agreement with previous reports, EB treatment elicited a modest midafternoon LH surge, as indicated by elevated LH levels at 1600 h in saline treated controls (Figure 16, lower pannel; $p < 0.05$; P. Kalra et al., 1972; S. Kalra and Kalra, 1979). In these rats, regardless of the time of administration, naloxone evoked a significant rise in serum LH levels. Elevations in serum LH levels over the control values varied between 48% and 73% before and after the spontaneous LH surge. At 1600 h, during the EB-induced LH surge, the naloxone-induced LH rise was not significantly different from that at other times studied.

As expected, P treatment advanced and amplified the LH surge in EB-primed rats (P. Kalra et al., 1972). A rise in serum LH levels was evident as early as 1400 h. Thereafter, LH levels peaked rapidly at 1600 h and decreased progressively at 1800 h and 2000 h. Also, P treatment markedly altered the naloxone-induced LH response. Within two hours of P treatment, the naloxone-induced LH response was significantly higher than that seen at this time in EB-treated rats (Figure 16) or before P treatment at 1000 h ($p < 0.05$). However, during the rising phase at 1400 h, the peak phase at 1600 h, and the receding phase at 1800 h of the LH surge, naloxone failed to stimulate any further

increase in LH release. The ability of naloxone to elicit LH release was again evident at the end of the LH surge at 2000 h. The LH response at this time was quite similar to that seen before the onset of the LH surge.

The Effects of P on Naloxone - Induced LH Release on Proestrus

The injection of P on proestrous morning advanced the midafternoon LH surge (Figure 17). Serum LH concentrations in P injected rats were significantly elevated by 1400 h, while oil-injected rats showed no evidence of an LH surge at this time. Secretion of IH increased further at 1600 h in these P-treated rats ($p < 0.05$). However, naloxone-induced LH responses observed in the oil-injected controls at 1400 h (Figure 17) or 1600 h (Figure 16) were abolished by P pretreatment.

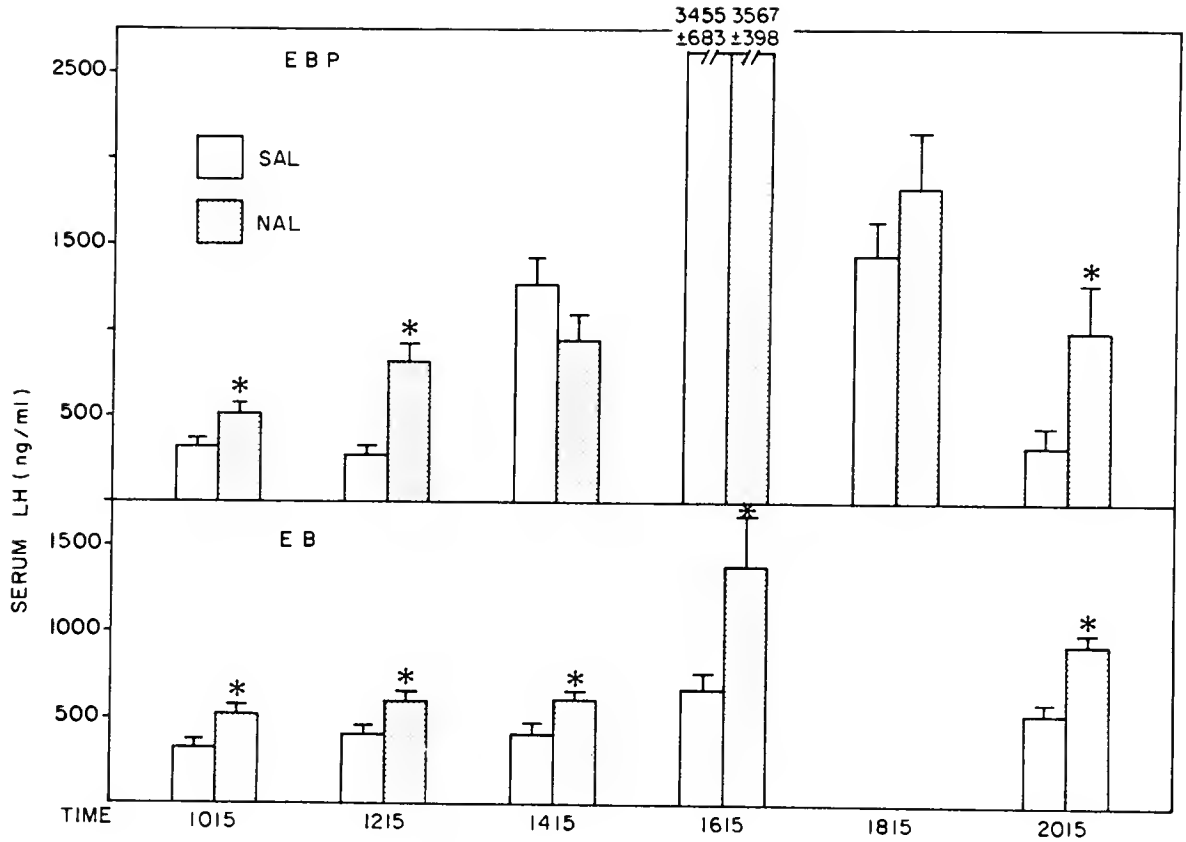

Figure 16: The effects of naloxone (2 mg/kg) on serum LH in ovariectomized rats treated with EB (lower panel) or EB plus P (upper panel).

The time of death on day 2 after EB treatment, noted on the abscissa, was 15 minutes after naloxone (NAL) or saline (SAL) injection. The EB group treated with naloxone or saline at 1000 h is also presented as a control group in the EBP panel, since it did not receive P on day 2 at 1000 h. * denotes $p < 0.05$ vs. saline control at the same injection time.

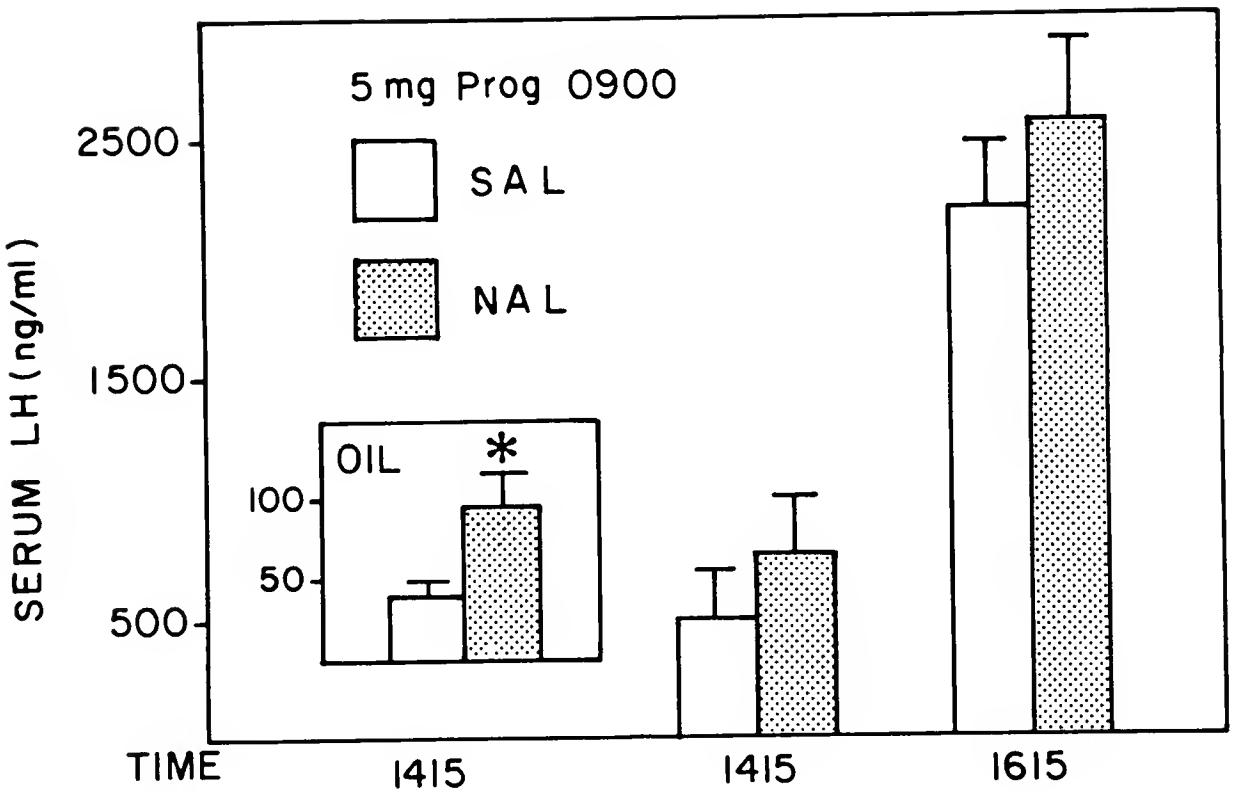

Figure 17: Effects of P (5 mg Prog) on naloxone (2 mg/kg) - induced LH secretion on proestrus.

The time of death, noted on the abscissa, was 15 minutes after naloxone or saline injection. Controls (inset) were treated with oil rather than P at 0900 h and received saline or naloxone at 1400 h. * denotes $p < 0.05$ vs. saline control at the same injection time.

Discussion

These studies clearly demonstrate that naloxone administration can readily stimulate LH release at all stages of the estrous cycle. Since the primary action of naloxone is believed to involve the displacement of the EOP from their receptors, it seems likely that this action led to the stimulation of LH release in this study (Meites et al., 1979; Cicero, 1980b; S. Kalra et al., 1980). This observation is in line with the view that during the estrous cycle, EOP containing neurons, located within the hypothalamus, exert a tonic inhibitory influence on LH release (Rotsztejn et al., 1978, S. Kalra, 1981).

Blank et al. (1979) were able to elicit LH release with naloxone (at 2.5 mg/kg) in prepubertal female rats. In adult cycling rats, they failed to observe a stimulatory LH response after a similar naloxone treatment. While the reasons for the disparity between these data and the work of Blank et al. (1979) are not apparent, it should be noted that the basal serum LH levels in the previous work were two to three times higher than the levels determined in this experiment. Whether this high basal LH secretion rendered the adult rats unresponsive to naloxone is uncertain. Interestingly, Ieiri et al. (1980b) also found that a single injection of naloxone on the afternoon of proestrus slightly prolonged the duration of the LH surge.

Quite unexpectedly, the LH response after naloxone treatment between diestrus II and just before the preovulatory LH release were similar. This finding is interesting because it suggests that factors other than the responsiveness of the pituitary to LHRH, which increases progressively as a function of E2 secretion during this period, may be operative after naloxone treatment (S. Kalra and Kalra, 1974; Libertun et al., 1974; Martin et al., 1974; Gabriel et al., 1983). Evidence suggests that naloxone treatment induces LHRH output from the hypothalamus (S. Kalra, 1981; Wilkes and Yen, 1981; Blank and Roberts, 1982). Therefore, it is likely that the uniform naloxone-induced LH responses coincident with the progressive enhancement in pituitary responsiveness to LHRH result from a corresponding gradual decrease in naloxone-induced LHRH release. Accordingly, it is predictable that late on diestrus II, when the pituitary sensitivity to LHRH is low, naloxone may have elicited LHRH output at considerably higher levels than later, at any time before the preovulatory LH release on proestrus. This interesting possibility of a gradual decrease in naloxone-induced LHRH release as estrogen secretion increases between diestrus II and proestrus would require further documentation by direct measurement of LHRH levels in the hypophysial portal plasma. It could indicate that over the 36 hour period approaching the proestrous LH surge that there is a gradual decrease in the activity of ECP-containing neurons controlling LH release.

In immature 22-day-old rats, EB treatment blocked the stimulation of LH release by naloxone (Blank et al., 1979). Similarly, Blank et al. (1980) reported that up to 4 hours after an EB injection, naloxone was ineffective in stimulating LH release in ovariectomized adult rats. However, as this study indicates, two days after EB treatment, the ability of naloxone to stimulate LH secretion returns. The injection of EB has been shown to exert biphasic effects on pituitary responsiveness to LHRH in ovariectomized rats (Libertun et al., 1974). After EB treatment, there is initially a suppression for up to 6 hours, followed by an enhanced LH response to LHRH injection. Thus the apparent contradictory observations of Blank et al. (1979 and 1980) and this study can be reconciled on the basis of changing patterns of pituitary responsiveness after EB injection. However this mode of biphasic change in pituitary responsiveness does not explain the uniform LH responses during the period between 2015 h on diestrus II and 1415 h on proestrus in cycling rats. At present it cannot be precluded that the rise in estrogen secretion may attenuate naloxone-induced LHRH release over this interval.

With respect to the effects of P, the data are consistent with the observation that P advances and amplifies the LH surge in EB-primed ovariectomized and proestrus rats (F. Kalra et al., 1972; Redmond, 1968). This study has further

found that two hours after P injection, the naloxone-induced LH response was greatly enhanced in EB-primed rats. This may be partly due to increased availability of a pool of releasable LHRH and enhanced pituitary sensitivity to LHRH at this time (Cooper et al., 1973; Aiyer et al., 1976; Simpkins and Kalra, 1980). Interestingly, as the LH surge began in these P-treated (EB-primed or proestrus) rats, naloxone was no longer able to elicit LH release. This refractory period began 4 hours after P injection and lasted for almost 8 hours when LH was being continuously released at high rates. The failure of naloxone to stimulate LH release cannot be entirely due to the possibility that the hypothalamic-pituitary-LH axis is responding maximally (Blake and Garner, 1980), because the refractory period was seen even 4 hours after P injection, when LH secretion was clearly not maximal. It should, however, be noted that the endogenous P secretion that follows the LH rise on proestrus failed to suppress the naloxone-induced LH response (S. Kalra and Kalra, 1974a). Under these conditions, either the optimal time course of P action was not allowed before the naloxone test or the endogenous P secretion failed to reach the effective concentration attained after exogenous P administration.

In the absence of P treatment, naloxone was highly effective in eliciting LH release throughout the LH surge on proestrus and in EB-treated rats. These observations are

interesting in two ways. First, they suggest that considerable amounts of biologically active LHRH were not being secreted during the LH surge on proestrus or in EB-treated rats, and this was not the case after P treatment. Second, the fact that naloxone can evoke further stimulation of LH release indicates that a considerable degree of inhibitory influence of EOP was present during the surge in proestrus and EB-primed rats and not in P-treated rats. It appears, therefore, that P treatment may serve to curb the inhibitory influence of EOP to allow LHRH output to occur at an optimal rate for amplification of the LH surge. The mechanism by which P exerts these effects is not known.

In summary, these studies show that naloxone can elicit LH release in intact and estrogen-treated ovariectomized rats. Further, estrogens may alter this LH response. In some circumstances, the alteration of estrogen may reflect pituitary sensitivity to LHRH at the time of naloxone treatment, while in others it may reflect the degree of inhibitory influence of EOP on LHRH neurons in the hypothalamus. While some degree of inhibitory influence continues to exist during the LH surge on proestrus and in EB-treated rats, P treatment appears to abolish this influence in association with amplification of the LH surge. It is possible that the action of exogenous P in advancing and magnifying the LH surge may in part be due to alterations in opioid activity before and during the hypersecretion of LH.

CHAPTER VIII
A DECLINE IN ENDOGENOUS OPIOID INFLUENCE DURING THE STEROID - INDUCED HYPERSECRETION OF LUTEINIZING HORMONE IN THE RAT

Introduction

A variety of anatomical, physiological and pharmacological evidence indicates a role for EOP in the regulation of LH secretion (Meites et al., 1979; Cicero, 1980b; S. Kalra et al., 1980; Watson et al., 1980). While morphine or EOP can suppress LH secretion, the significance of these pharmacological effects is uncertain (Cicero et al., 1976; Kinoshita et al., 1981; Leadem and Kalra, 1983). However the capacity of the narcotic antagonist, naloxone, when administered alone to elicit LH secretion suggests a tonic inhibitory role for EOP (Bruni et al., 1977; Cicero et al., 1981). Further the magnitude of the LH secretory response to naloxone may serve as an indication of the level of ongoing opioid agonist activity exerted at the level of the hypothalamic-pituitary-LH axis (Cicero et al., 1983b).

As was suggested in the previous chapter, EOP-containing neurons appear to tonically inhibit LH secretion throughout the estrous cycle of the rat, and ovarian steroids appear to modulate this inhibitory activity. To further evaluate the role of EOP in the phasic secretion of LH, this chapter

examines the LH secretory response to a wide range of naloxone dosages, prior to and during the period of LH hypersecretion seen following the administration of EB or EB plus P to ovariectomized rats.

Experimental

This study consists of two experiments, both of which employ models routinely used to simulate preovulatory LH release in the female rat (as described in General Materials and Methods). Two weeks after ovariectomy, rats receive 7.5 micrograms EB in oil (EB priming) at 1000 h. After two days of EB treatment, a diffuse midafternoon LH surge ensues with peak LH levels achieved around 1600 h (Legan et al., 1975). The administration of P (5 mg., s.c. in oil) 48 hours after EB treatment (day 2, 1000 h, EBP priming) advances the onset and increases the magnitude of the resultant LH surge (P. Kalra et al., 1972). The following three sampling times were used in these EBP-treated rats:

1. during the basal LH secretion seen prior to the onset of the LH surge at 1200 h;
2. at the onset of the LH surge at 1400 h; and
3. during the period of maximal LH titers at 1530 h.

Additionally, for comparison, rats treated with EB alone were sampled at 1600 h during the peak LH response to this treatment regimen.

In the first experiment, groups of EB- and EBP-primed rats were injected with saline vehicle or naloxone HCl dissolved in saline at dosages ranging from 0.1 to 15.0 mg/kg B.W. Blood was collected by decapitation at exactly 15 minutes after injection. The dose range of naloxone and the sampling time were chosen on the basis of work done in this and other laboratories (Cicero et al., 1981; S. Kalra and Simpkins, 1981).

In the second experiment, the pituitary responsiveness to LHRH injections (75 ng/100 gm B.W., s.c.) was evaluated in rats pretreated with EB or EBP as described above. Blood samples (750 microliters) were obtained by cardiac puncture prior to and 30 minutes after LHRH injection. Light ether exposure was used as an anesthetic. The dosage of LHRH, as well as the sampling interval, were based on previous work done by in this and other laboratories (Chapter V; Lu et al., 1980).

Results

The effects of gonadal steroid treatment on serum LH concentrations in ovariectomized rats are shown in Figure 18. In EBP-treated rats treated with saline, LH concentrations at all three time intervals were significantly different from one another. Concentrations of LH increased progressively between 1200 h and 1530 h following P injection to EB-primed rats. Further, LH levels

at 1530 h in EBP-treated rats were more than 4-times higher than EB-treated rats at a similar time (1600 h; $p < 0.05$).

The effects of naloxone on LH secretion during the hormone surge induced by gonadal steroids in ovariectomized rats are also shown in Figure 18. All dosages of naloxone employed from 0.1 through 4.5 mg/kg B.W. stimulated LH release prior to the LH surge in EBP-treated rats at 1200 h and during the LH surge in EB-primed rats at 1600 h. The lowest effective dose (0.1 mg/kg B.W.) stimulated LH secretion 73% and 56% in EBP-treated rats at 1200 h and EB-treated rats at 1600 h, respectively ($p < 0.05$). In contrast, during the LH hypersecretion induced by EBP priming to ovariectomized rats, higher dosages of naloxone were required to stimulate LH levels further. In EBP-treated rats at 1400, only the highest dose of naloxone (15 mg/kg B.W.) significantly increased LH levels while the 4.5 mg/kg B.W. dose of naloxone was required to significantly stimulate LH levels at 1530 h.

The effects of LHRH administration (75 ng/100 g. B.W., s.c.) on LH secretion in gonadal steroid primed rats are shown in Table 4. Prior to LHRH injection, LH concentrations were elevated in EB-treated rats at 1600 h and in EBP-treated rats at 1400 h and 1530 h relative to EBP-treated rats at 1200 h ($p < 0.05$). Paired t-analysis revealed a significant stimulation of LH secretion after LHRH injection in all 4 gonadal steroid treatment groups.

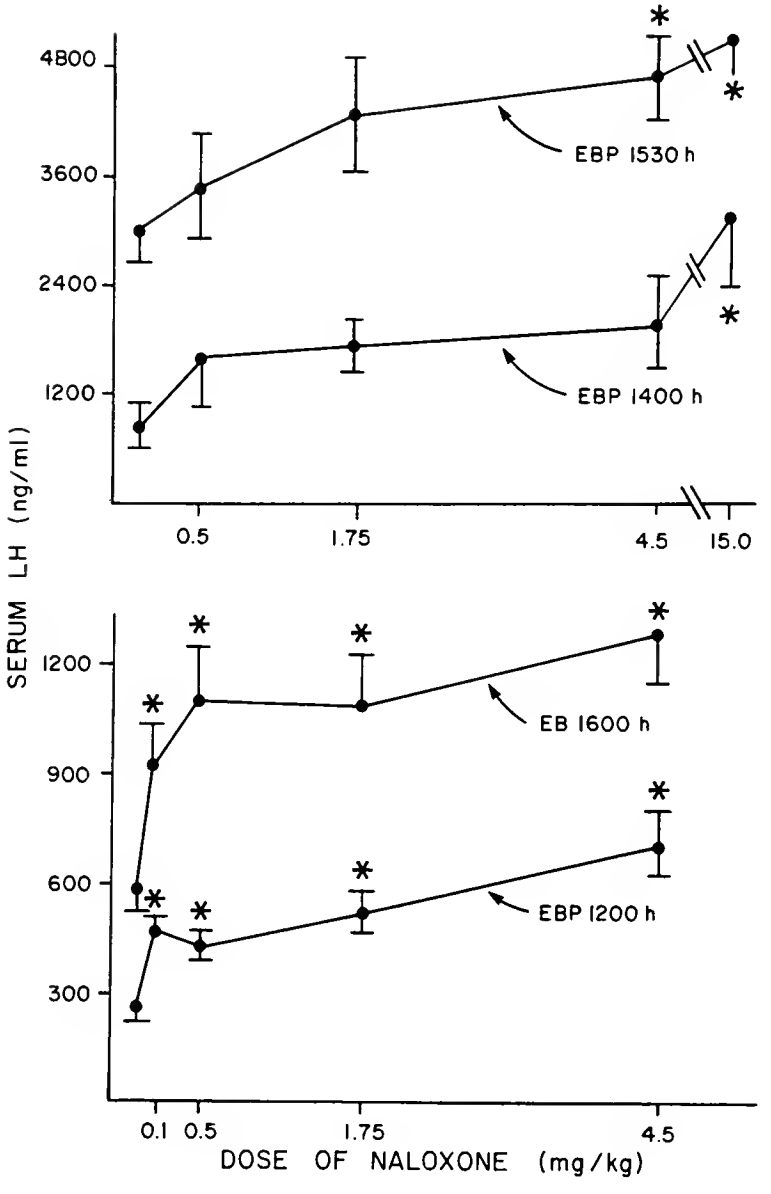

Figure 18: The effects of naloxone injection on LH secretion in ovariectomized rats receiving EE or EBP treatment.

Data are expressed in terms of LH-RP-1 (LH-RP-1 = 61 X LH-RP-1) * denotes $p < 0.05$ vs saline.

The magnitude of the LH secretory response to LHRH (delta LH) was similar in EBP-treated rats at 1200 h and 1400 h and in EB-treated rats at 1600 h. However, the LH response to LHRH injection in rats treated with EB plus P at 1530 h was greater than the response seen in all other treatment groups ($p < 0.05$).

TABLE 4

Effects of LHRH (75 ng/100 gm B.W., s.c.) on LH secretion in ovariectomized steroid treated rats.

Group	Pre-LHRH	Post-LHRH	delta-LHRH
	LH (ng/ml serum)		
EBP 1200	206 ± 27	1372 ± 190	1206 ± 198
EBP 1400	358 ± 28[1]	1162 ± 184	803 ± 184
EBP 1530	2006 ± 406[1]	5551 ± 1329[1]	3538 ± 1055[1]
EB 1600	375 ± 25[1]	1311 ± 153	934 ± 153

Data are expressed in terms of LH-RP-1 (LH-RP-1 = 61 X LH-RP-2).
[1] denotes $p < 0.05$ vs. EBP 1200 h.

Discussion

The results of the present study provide clear evidence that during the period of LH hypersecretion induced in ovariectomized rats by the sequential administration of EB plus P, a decline in the inhibitory influence of EOP on LH secretion occurs. This is evidenced by the marked decline in the ability of naloxone to elicit LH secretion during this steroid-induced LH surge. The present study further indicates that the decline in the LH secretory response to naloxone cannot be fully explained by changes in the response of the pituitary to LHRH, or to increased variability in LH levels inherent to the onset of the LH hypersecretory state.

As seen in Table 4, LHRH administration to EB-treated rats (1600 h) caused a marked elevation in LH concentrations, and P administration to these EB-primed rats (1530 h) further augmented the pituitary response to LHRH, as has been noted previously (Aiyer et al., 1976). Additionally, during the progression of the EBP-induced LH surge, the response to LHRH was stable at 1200 h and 1400 h, and increased at 1530 h, during the period of the peak LH response to gonadal steroid priming. These results suggest that the heightened pituitary responsiveness to LHRH at the apex of the LH surge, as has been noted by others, is a consequence of LHRH self-priming (Blake and Garner, 1980) and the direct action of gonadal steroids on the pituitary.

Regardless of the mechanism of this enhanced responsiveness, it is clear that the diminished ability of naloxone to stimulate LH release during the EBP-induced LH surge is not a consequence of a hypophyseal deficit.

In EB-treated rats at 1600 h and in EBP-treated rats at 1200 h, the LH secretory mechanism is extremely sensitive to naloxone and comparable to that previously reported in male rats (Cicero et al., 1981). Naloxone appears to stimulate LH release by blocking the ongoing actions of EOP (Bruni et al., 1977; Cicero et al., 1981; S. Kalra and Simpkins, 1981). The extreme sensitivity of these animals to naloxone would suggest that ongoing EOP neuronal activity plays an inhibitory role in the period preceding the LH surge induced by EBP treatment as well as in dampening the magnitude of the LH surge induced by EB priming alone. These observations as well as the findings of the previous chapter that naloxone can stimulate LH secretion throughout the estrous cycle would support such an inhibitory role of EOP on LH release in the female rat.

During the hypersecretion of LH induced by P administration to EB-primed rats, the influence of EOP on LH secretion diminishes, as is evident by the relative insensitivity of the LH secretory mechanism to naloxone injection. The high dose of naloxone required to stimulate LH secretion in EBP-primed rats at 1400 h (15 mg/kg B.W.) and at 1530 h (4.5 mg/kg B.W.) suggests that the LH

secretory response observed in these animals may not be due to the antagonism of opioid receptors. In the dose range of 5 through 20 mg/kg B.W., naloxone can have non-opioid and opiate agonist activity (Sawynok et al., 1979). However, a caution in interpreting the response to naloxone during LH hypersecretion should be presented. Since the onset and magnitude of steroid-induced LH surges vary among animals, higher variability in LH levels would be expected during the LH surge. Consequently, a proportionately larger response to naloxone would be required to discern a significant increase in serum levels of the hormone. However, this variability alone cannot account for the lack of response to naloxone during the EBP-induced LH surge. Only small changes in the mean LH response were observed during the LH surge at naloxone dosages (0.1 and 0.5 mg/kg B.W.) which caused nearly maximal LH secretory responses in EB-treated rats and EBP-treated rats at 1200 h. Furthermore, other LH secretory states which have an inherently high variation in mean LH levels, such as the acutely orchidectomized male rat, are highly sensitive to naloxone-induced LH secretion (Cicero et al., 1981)

In EBP-treated rats, the resulting LH surge is earlier in onset, higher in magnitude and shorter in duration than the LH surge observed in EB-treated rats (P. Kalra et al., 1972; Aiyer et al., 1976). The findings of this and the previous chapter indicate that EDP may be involved in determining

these LH secretory responses to gonadal steroids. For a 4 hour period following P injection to EB-primed ovariectomized rats, ECP inhibitory influence on LH secretion persists and may be enhanced. During the ascending and peak phase of EBP-induced LH hypersecretion, ECP influence is lacking, suggesting that associated with this LH surge is a decline in ECP neuronal activity or the responsiveness of its post-synaptic effectors. Further, the decline in the LH surge induced by EBP-treatment is associated with a reinitiation of naloxone-induced LH secretion, suggesting that ECP may be involved also in the termination of LH hypersecretion (Chapter VII). In contrast, EOP neuronal systems appear to be active during the entire extent of the EB-induced LH surge. It is reasonable to assume, therefore, that EOP may be involved in the late onset, and low magnitude of EB-induced LH hypersecretion. Methods of measuring the release of various ECP prior to and during steroid-induced LH hypersecretion are clearly needed to test this hypothesis.

CHAPTER IX
THE EFFECTS OF CHRONIC MORPHINE TREATMENT ON THE FEEDBACK ACTIONS OF ESTROGEN ON GONADOTROPIN SECRETION IN OVARIECTOMIZED RATS

Introduction

The feedback effects of the ovarian steroid, E2, on the gonadotropins LH and FSH, are coordinated at the levels of the hypothalamus and pituitary (Fink, 1979; S. Kalra and Kalra, 1983). E2 displays both inhibitory and stimulatory effects on gonadotropin secretion. Inhibitory, or negative feedback effects, are seen during periods of basal LH secretion throughout the estrous cycle or immediately after the injection of E2 to ovariectomized rats, while stimulatory, or positive feedback effects, are witnessed after periods of increased follicular estrogen secretifrom diestrus through on proestrus or after more than 48 hours of sustained E2 exposure to ovariectomized rats (Vilchez-Martinez et al., 1974; Legan et al., 1975; Fink, 1979; S. Kalra and Kalra, 1983). In the ovariectomized rat, the positive feedback effects of estrogen are expressed as a daily signal for midafternoon LH hypersecretion which ensues for several days if E2 titers are maintained at levels similar to or greater than E2 levels seen on proestrus (Legan et al., 1975). The neuronal mechanisms mediating this LH hypersecretion are only partially understood.

The localization of EOP in brain regions closely associated with steroid-concentrating neurons and LHRH-containing neurons has sparked an investigation of their role in the regulation of gonadotropin secretion (Meites et al., 1979; Cicero, 1980b; S. Kalra et al., 1980). It is not certain whether tissue levels of EOP in the hypothalamus change in response to alterations in the reproductive state of the female rat (Kumar et al., 1980; Knuth et al., 1984). A large body of pharmacologic evidence, however, indicates that EOP act to inhibit LH secretion and possibly mediate the feedback effects of E2 (S. Kalra, 1981; S. Kalra and Simpkins, 1981, Sylvester et al., 1982).

As was shown in the two previous chapters, EOP appear to inhibit LH secretion throughout the estrous cycle, and may participate in the advanced onset and increased magnitude of the LH surge seen after P treatment to proestrous and EB-primed ovariectomized rats. Additionally, it was shown that chronic morphine treatment to castrated male rats enhanced the negative feedback effects of T on gonadotropin secretion (Chapters IV through VI). This chapter concludes the experimental work presented in this dissertation by investigating the feedback effects of E2 on gonadotropin release in ovariectomized rats treated with morphine.

Experimental

In these studies ovariectomized rats were treated with morphine and E2 implants or appropriate control treatments as described in the General Materials and Methods section of this dissertation. All treatments were initiated two weeks after ovariectomy.

Experiment 1

This study evaluated the inhibitory and stimulatory effects of E2 on LH and FSH secretion in ovariectomized rats pretreated with morphine or placebo pellets. Ovariectomized rats were given morphine or placebo pellets, followed two days later at 1000 h by two additional implants. At the time of the second morphine or placebo pellets, groups of rats received E implants at one of three dosages:

1. 5 mm long tubes packed with crystalline E2 diluted with cholesterol on a (weight:weight) basis of 1:1; hereafter referred to as 5 mm E2 (1:1);
2. 5 mm long tubes packed with crystalline E2; hereafter referred to as 5 mm E2; and
3. 10 mm long tubes packed with crystalline E2; hereafter referred to as 10 mm E2.

On the second day after E2 treatment, basal LH secretion is seen at 1000 h while LH hypersecretion is observed at 1600 h (Legan et al., 1975). Groups of E2-implanted rats treated with morphine or placebo pellets were killed at

these two times. Trunk blood was collected for serum LH and FSH analysis by RIA.

Experiment 2

Based on the results of Experiment 1, groups of ovariectomized rats were given morphine or placebo pellets plus one 5 mm E2 (1:1) implant as described in Experiment 1. To obtain a more detailed time course of the E2-induced gonadotropin surge, groups of animals were sacrificed at 1200 h, 1400 h, 1600 h, and 1800 h on the second day after E2 implantation. Serum from trunk blood was stored at -20° C. for later analysis of LH and FSH by RIA.

Experiment 3

An LH surge can be initiated for several days after the implantation of E2 capsules to ovariectomized rats if serum E2 levels are maintained elevated (Legan et al., 1975). In this experiment rats received morphine or placebo treatment plus one 5 mm E2 (1:1) implant as described in Experiment 1. Both morphine and E2 treatment were continued for an additional two days, however. At this time (after 6 days of morphine or placebo treatment and 4 days after receiving the E2 implant) groups of rats were killed at 1000 h and 1600 h. Trunk blood was collected for later serum LH and FSH assays.

Experiment 4

To determine whether chronic morphine treatment alone altered gonadotropin secretion in the absence of E2 treatment, groups of rats were treated with morphine or placebo pellets plus sham capsules. After 4 days of morphine or placebo treatment, groups of rats were killed by decapitation at 1000 h and 1600 h. Serum was stored at -20° C. for later serum hormone analysis.

Experiment 5

To determine whether chronic morphine treatment influenced the pituitary response to LHRH, groups of rats received morphine or placebo treatment plus 5 mm E2 (1:1) capsules as described in Experiment 1. On the second day after E2 treatment rats were given an injection of LHRH (75 ng/100 g. B.W., s.c.) at 1530 h. Blood samples (750 microliters) were obtained by cardiac puncture under light ether anesthesia prior to, and 30 minutes after LHRH injection. The dose of LHRH used and the sampling interval were based on earlier experiments (Chapters V and VIII; Lu et al., 1980). Serum was analyzed for LH by RIA.

Results

The inhibitory and stimulatory effects of three different E2 doses on LH and FSH release in placebo-implanted rats are shown in Figure 19. At 1000 h, serum LH levels were below 225 ng/ml in these placebo-treated, E2-implanted rats. This is comparable to LH levels shown in earlier chapters employing EB-treated ovariectomized rats (Figures 16 and 18). Midafternoon elevations in serum LH were evident in all three of these E2-implanted groups at 1600 h relative to 1000 h ($p < 0.05$). Of the three E2 doses used, the 10 mm E2 implants caused the largest (6-fold) increase in serum LH above those observed at 1000 h. The FSH response to E2 implantation in placebo-treated rats was less pronounced than that of LH (Figure 19, lower panel). Serum FSH levels at 1000 h in placebo-treated rats were inhibited by the 5 mm and 10 mm E2 implants compared to FSH levels seen following 5 mm E2 (1:1) implantation. In placebo-treated rats none of the E2 implants produced a significant elevation in midafternoon serum FSH concentrations.

The feedback effects of E2 on serum LH and FSH concentrations in the presence of chronic morphine treatment are also shown in Figure 19. Treatment with morphine further reduced LH levels at 1000 h by 65%, 58% and 50% in 5 mm E2 (1:1), 5 mm E2 and 10 mm E2-implanted rats, respectively ($p < 0.05$). Surprizingly, concurrent treatment with morphine plus E2 enhanced the midafternoon

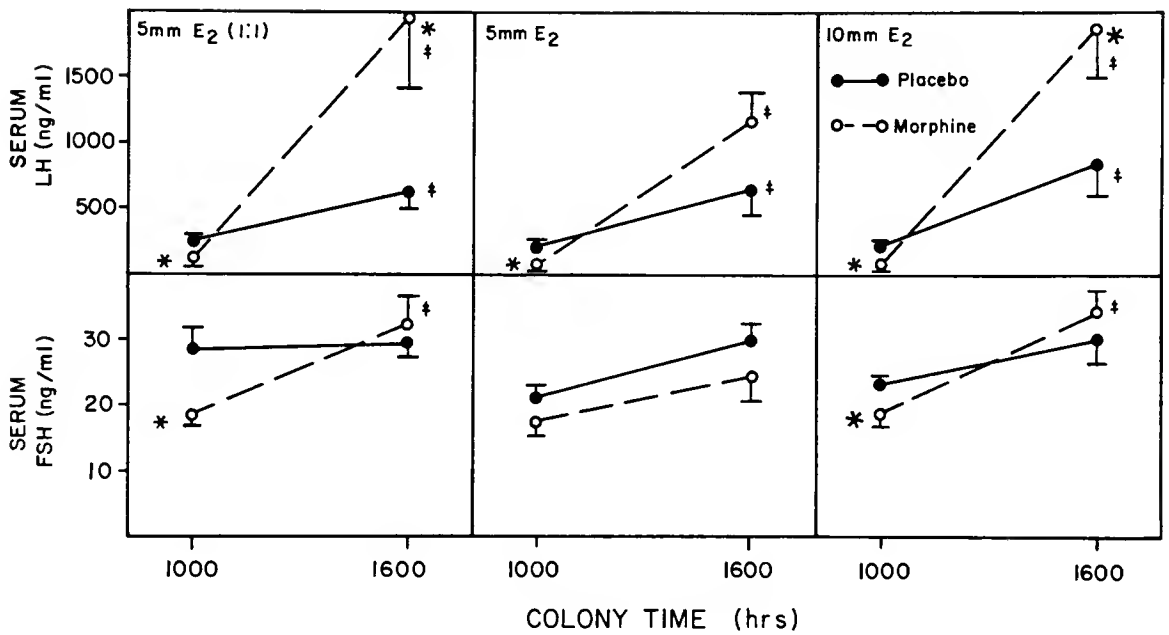

Figure 19: The effects of continuous morphine exposure and various doses of E2 on serum LH and FSH levels in ovariectomized rats.

Crystalline E2 or E2 diluted on a 1:1 (weight:weight) ratio with cholesterol was packed into tubes as shown in the figure. Rats were sacrificed at the times shown 4 days after morphine or placebo treatment and two days after E2 implantation. LH levels were determined using the LH-RP-2 reference standard and are expressed relative to the LH-RP-1 standard (LH-RP-1 = 61 X LH-RP-2). * denotes $p < 0.05$ vs. placebo treatment at the same time point; the dagger symbol denotes $p < 0.05$ vs. 1000 h.

hypersecretion of LH seen at 1600 h. Exposure to morphine enhanced LH levels 3-fold in 5 mm E2 (1:1)-treated rats and 2-fold in 10 mm E2-treated rats ($p < 0.05$) when compared to placebo-treated rats at this time. Although the 5 mm E2 implant caused a proportionately larger LH surge in morphine than in placebo-treated rats (greater than 12-fold vs. more than 4-fold, respectively), peak LH levels at 1600 hours were not significantly different from one another.

Chronic morphine treatment also altered the response of FSH to E2 exposure (Figure 19, lower panel). Levels of FSH were reduced relative to placebo-implanted controls at 1000 h in 5 mm E2 (1:1) and 10 mm E2-implanted rats, but not in the 5 mm E2-treated group ($p < 0.05$). Additionally, midafternoon FSH levels in these morphine-treated rats (1600) were significantly increased by the 5 mm E2 (1:1) and 10 mm E2 implants relative to morning values (1000 h).

Figure 20 displays the time course of the E2-induced LH and FSH surges in placebo and morphine-treated rats. Serum LH concentrations in placebo-implanted rats increased greater than 4-fold over the period from 1200 h to 1800 h ($p < 0.05$). Concentrations of FSH in the blood increased in a similar, though more gradual fashion, over this period (46%, $p < 0.05$). Chronic morphine exposure to these 5 mm E2 (1:1)-implanted rats appeared to advance the onset and augment the magnitude of the midafternoon gonadotropin surges. Compared to 1200 h, serum LH concentrations in

morphine-treated animals were increased earlier (at 1600 h) than similarly treated placebo-implanted rats. Serum LH levels seen in morphine-treated rats at 1600 h and 1800 h were twice placebo-implanted LH levels ($p < 0.05$). Chronic morphine treatment significantly reduced FSH levels prior to (1200 h) and augmented FSH levels during (1800 h) the surge induced by the 5 mm E2 (1:1) implant when compared to ovariectomized placebo-implanted rats.

After 4 days of 5 mm E2 (1:1) exposure, midafternoon (1600 h) hypersecretion of LH but not FSH was noted in placebo-treated rats relative to 1000 h (Figure 21, $p < 0.05$). While morphine treatment further reduced serum LH levels at 1000 h ($p < 0.05$), treatment with the opiate did not alter LH values at 1600 h. Further, chronic morphine did not effect FSH levels at these two times.

Serum LH and FSH levels in ovariectomized rats receiving 4 days morphine treatment are shown in Figure 22. LH concentrations in ovariectomized rats (placebo treatment) at 1000 h were 902 ± 33 ng/ml. At 1600 h these levels were decreased by 30% ($p < 0.05$). Serum FSH concentrations in ovariectomized rats at 1000 h (32.4 ± 1.2) were similar to those seen in the afternoon. After 4 days of exposure to morphine, LH concentrations at 1000 h were similar to placebo-implanted rats. Interestingly, morphine treatment stimulated LH levels at 1600 h 39% compared to morphine-treated rats at 1000 h, and 76% compared to placebo-treated rats at 1600 h ($p < 0.05$).

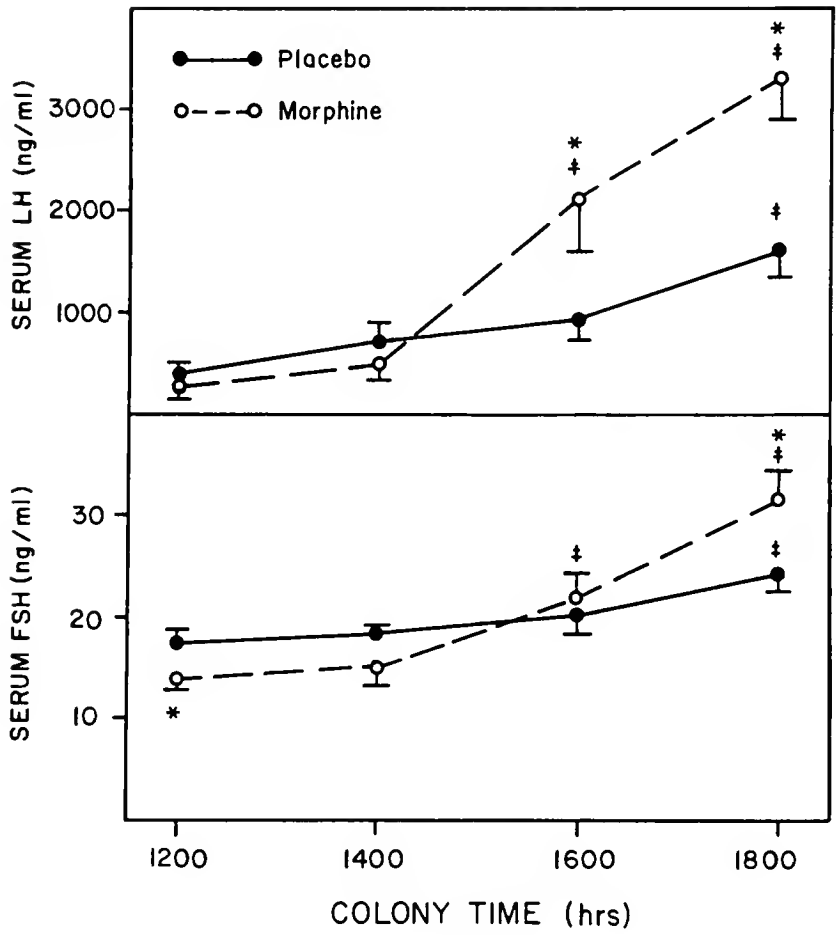

Figure 20: The effects of continuous morphine exposure on midafternoon LH and FSH hypersecretion induced by E2 implantation in ovariectomized rats.

Crystalline E2 was diluted on a 1:1 (weight:weight) ratio with cholesterol and packed into tubes 5 mm in length. Animals were sacrificed at the times indicated after 4 days of morphine or placebo treatment and 2 days after 5 mm E2 (1:1) implantation. LH levels were determined using the LH-RP-2 reference standard and are expressed relative to the LH-RP-1 standard (LH-RP-1 = 61 X LH-RP-2). * denotes $p < 0.05$ vs. placebo treatment at the same time point; the dagger symbol denotes $p < 0.05$ vs. 1200 h.

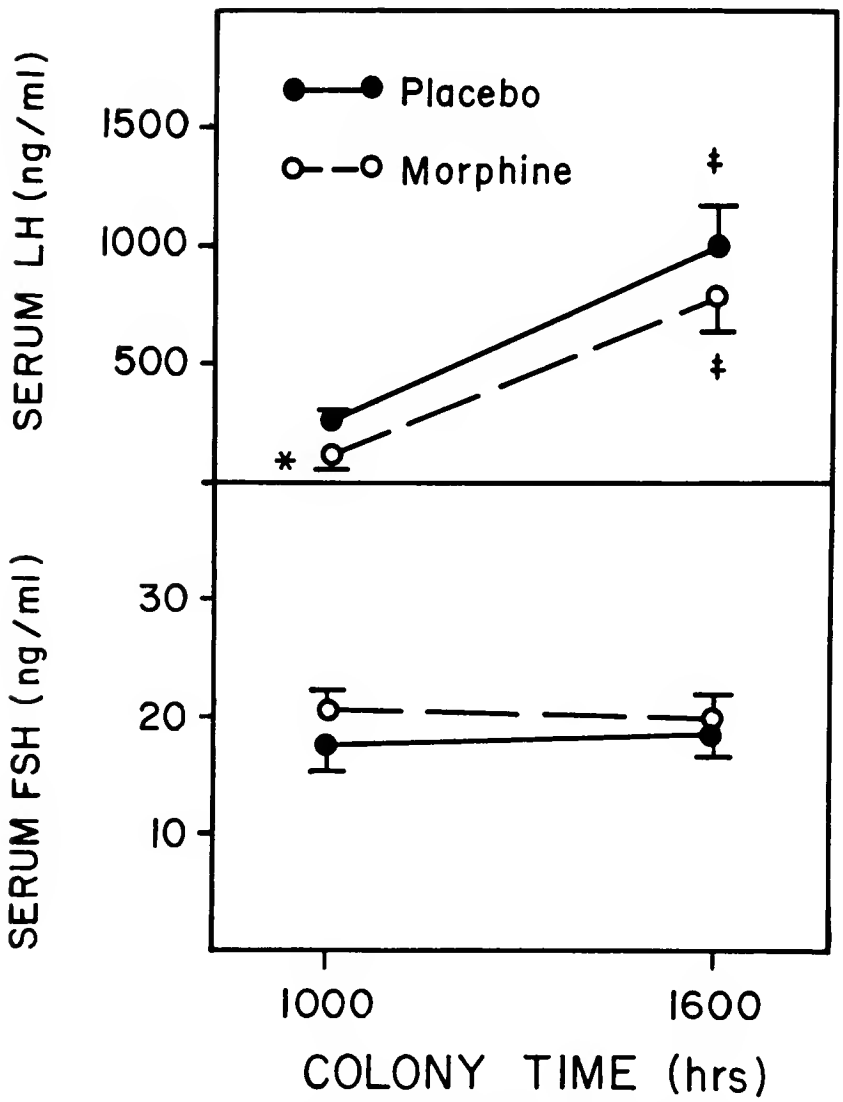

Figure 21: The effects of continuous morphine exposure on LH and FSH secretion in E2 implanted ovariectomized rats.

Crystalline E2 was diluted on a 1:1 (weight:weight) ratio with cholesterol and packed into tubes 5 mm in length. Animals were sacrificed at the times indicated after 6 days of morphine or placebo-treatment and 4 days after 5 mm E2 (1:1) implantation. LH levels were determined using the IH-RP-2 reference standard and are expressed relative to the LH-RP-1 standard (LH-RP-1 = 61 X IH-RP-2). * denotes $p < 0.05$ vs. placebo treatment at the same time point; the dagger symbol denotes $p < 0.05$ vs. 1000 h.

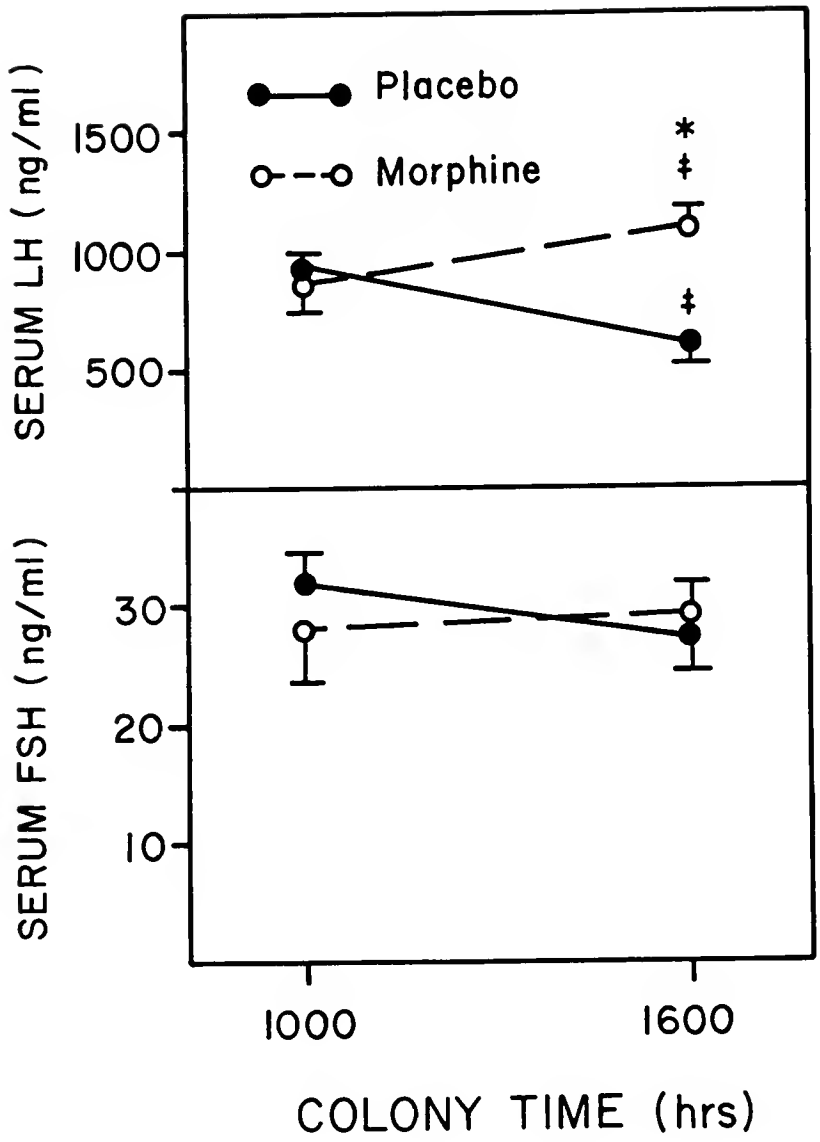

Figure 22: The effects of continuous morphine exposure on LH and FSH secretion in ovariectomized rats.

Animals were killed at the times indicated after 4 days of morphine or placebo treatment. LH levels were determined using the LH-RP-2 reference standard and are expressed relative to the LH-RP-1 standard (LH-RP-1 = 61 X LH-RP-2). * denotes $p < 0.05$ vs. placebo treatment at the same time point; the dagger symbol denotes $p < 0.05$ vs. 1000 h.

The effects of LHRH (75 ng/100 gm B.W., s.c.) injection on LH secretion in 5 mm E2 (1:1)-treated ovariectomized rats given morphine or placebo implants are shown in Table 5. Serum LH concentrations prior to LHRH injection in these 5 mm E2 (1:1)-treated rats were similar to LH levels in EB-treated rats at 1600 h (Table 4). LHRH injection significantly increased LH levels in both treatment groups ($p < 0.01$). In this study, morphine treatment did not appear to influence LH levels before or after LHRH injection.

TABLE 5

The Effects of LHRH on LH Hypersecretion in E2-treated Ovariectomized rats.

Treatment:	Pre-LHRH	Post-LHRH	delta-LHRH
	LH (mg/ml serum)		
E2	386 ± 30	2196 ± 399	1488 ± 195
E2 + M.	383 ± 53	2574 ± 606	2191 ± 585

LH concentrations were determined using the IH-RP-2 reference standard and are expressed relative to the LH-RP-1 standard (LH-RP-2 = 61 X LH-RP-2).

Discussion

The present study reveals an interaction between morphine and E2 on the feedback control of gonadotropin secretion in the ovariectomized rat. In the presence of chronic morphine treatment both the inhibitory and stimulatory effects of E2 implantation on LH and FSH secretion are potentiated. Since the effects of opiates on gonadotropin secretion are mediated at a site within the central nervous system (Table 5; Cicero, 1980b; S. Kalra, 1981; Wiesner et al., 1984) and EOP likely exert effects similar to that of morphine (Leadem and Kalra, 1983), the present study argues for an important role of EOP-containing neurons in the regulation of the cyclic release of LH and FSH.

The ability of E2 to inhibit LH release was enhanced by concurrent treatment with morphine. Immediately after the administration of estrogens to ovariectomized rats, a decline in LHRH release combined with a decline in pituitary responsiveness to the decapeptide causes a rapid decline in LH levels (Vilchez-Martinez et al., 1974; Fink, 1979). During basal LH release seen 48 hours after estrogen treatment, pituitary responsiveness to LHRH is enhanced, however (Vilchez-Martinez et al., 1974). The negative feedback of E2 at this time is likely due to an inhibition of LHRH release, and it would appear that morphine enhances this effect. This interaction between morphine and E2 in

suppressing LH release is not merely additive, since after 4 days of treatment with the opiate there was no difference in LH levels between morphine and placebo-treated, sham-implanted (ovariectomized) controls at 1000 h (Figure 22). These findings are similar to the observations of Chapters IV through VI that while ineffective in inhibiting LH release on its own, chronic morphine treatment enhances the feedback sensitivity of T in male rats.

The lack of an effect of morphine in altering LH secretion in gonadectomized male or female rats is of interest since it indicates that the gonadal steroids are required to maintain the responsiveness of the neuroendocrine substrates mediating the LH response to opiates. This castration-induced loss in the LH response to opiates has been noted in Chapter 4 and by other investigators, although the mechanism of this response has not been determined (Cicero et al., 1982a; Bhanot and Wilkinson, 1983). Gonadal steroids do not appear to modify opiate receptor numbers (Cicero et al., 1983a). Further, cyclic fluctuations in gonadal steroids in intact female rats, and physiological doses of gonadal steroids in ovariectomized rats do not consistently modify EOP levels in the hypothalamus (Kumar et al., 1980; Wardlaw et al., 1980; Knuth et al., 1984). In monkeys however, beta-endorphin release into hypophyseal portal blood is clearly steroid dependent (Ferin et al., 1984). Finally, the interaction

between morphine and T in inhibiting LH release is not reflected by changes in the metabolism of DA, NE, or 5HT in the MBH or POA-AH (Chapter IV).

The observation that in the presence of chronic morphine treatment the stimulatory effects of E2 on LH release are enhanced is most surprising. It is well known that when given acutely, opiates block ovulation, and preovulatory and gonadal steroid-induced LH hypersecretion (Barraclough and Sawyer, 1955; Pang et al., 1977; S. Kalra and Simpkins, 1981). It is possible that chronic morphine treatment reveals a stimulatory opiate component on LH secretion in these E2-treated rats. This stimulatory effect of morphine on afternoon LH release was evident even in the absence of E2 treatment to ovariectomized rats (Figure 21). Other neurotransmitters, such as DA and NE have been shown to display both stimulatory and inhibitory effects on LH release (Drouva and Gallo, 1976a; Rotsztejn et al., 1977; Gallo and Drouva, 1979). Additionally, several investigators have observed stimulatory effects of opioids on LH release, particularly at low dosages, but they have not attempted to explain these effects (Pang et al., 1977; Cicero et al., 1980; Motta and Martini, 1982; Sylvester et al., 1982; Leadem and Kalra, 1983; Mittler et al., 1983; Wilkinson and Bhanot, 1983). Piva et al. (1984) have suggested that the stimulatory effects of intraventricularly administered morphine may be due to an opiate recepter

different from the site which mediates the inhibitory effects of morphine on LH release.

The LH surge in morphine dependent, E2-implanted rats was enhanced in magnitude and advanced in onset (Figure 20). It is well documented that P administration advances the time of onset and increases the magnitude of LH hypersecretion seen on proestrus or after E2 treatment to ovariectomized rats (Redmond, 1968; P. Kalra et al., 1972). Thus, chronic morphine treatment may have resulted in the same neuronal alteration as seen after P exposure. As was shown in Chapter VIII, EOP inhibition of LH secretion, present two hours after P injection to EE-treated rats, was diminished from 4 to 6 hours after P injection to these animals. In this sense, the morphine treatment may have stimulated receptors normally activated by EOP after P treatment. In morphine-treated, E2-implanted rats however, opiate receptors were being continuously stimulated during the interval that LH hypersecretion was occuring. Whether morphine acted to stimulate LH release at this time, or if its inhibitory effects were transiently removed is not known.

The interaction between E2 and morphine in inhibiting LH persisted for at least 4 days of E2 exposure (Figure 22). However, while the stimulatory effects of E2 alone persisted for 4 days, at this time morphine was no longer able to enhance this midafternoon LH surge.

While ovarian steroids clearly influence FSH release, their effects on FSH are less pronounced than on LH (Mahesh et al., 1975; Fink, 1979). This supports the role of other ovarian factors regulating this gonadotropin (Lumpkin et al., 1984). In this study E2 was also less effective in modulating FSH levels. While chronic morphine treatment produced smaller changes in FSH levels compared to LH, its influence was apparent. Morphine stimulated both positive and negative feedback responses at E2 doses which were themselves unable to alter FSH release. Like LH, the time-course and magnitude of the E2-induced FSH surge was both advanced and amplified, suggesting that the effects of morphine and E2 on FSH release may be mediated by the same neural mechanisms which regulate LH release. However, neuronal factors other than LHRH may ultimately mediate this effects of morphine and E2 on FSH release (McCann et al., 1983).

In conclusion, these studies indicate that chronic morphine treatment enhances the negative and positive feedback effects of E2 on gonadotropin release. This would suggest an important role for EOP in modulating the feedback actions of ovarian steroids. The existence of both stimulatory and inhibitory influences of morphine on E2 feedback over such a brief time interval (6 hours) indicates that the modulation of gonadotropin secretion by EOP is more complex than previously thought.

CHAPTER X
GENERAL DISCUSSION

The present work evaluated the interaction of opioids with gonadal steroids in their regulation of LH and FSH secretion in the rat. The individual experiments were organized into 6 studies - three in the male and three in the female. The major findings drawn from these studies are as follows.

1. Chronic morphine treatment, while unable to inhibit gonadotropin release alone, enhances the negative feedback sensitivity of T on gonadotropin release in rats orchidectomized two weeks previously.

This extends previous findings that castration results in a loss of the inhibitory effects of opioids on LH release, and that EOP act to inhibit the release of LH and mediate the feedback effects of gonadal steroids (Cicero et al., 1982a; Van Vugt et al., 1982; Bhanot and Wilkinson, 1983). The present studies suggest that in the presence of opioid receptor stimulation the central feedback inhibition of T on LH secretion is enhanced. In this respect, EOP may serve as the set point or gonadostat for T feedback.

It must be noted that chronic treatment with morphine serves as a pharmacological probe to elucidate the normal

function of EOP-containing neurons. Opiate dependence effects many neuronal systems in addition to the neuroendocrine substrates determining LH release. It cannot be assumed that the pattern of EOP release resembles that of continuous opiate receptor stimulation with morphine. Yet, this criticism can be argued for any pharmacological study of EOP-containing neurons, including acute opioid administration. Since gonadal steroids influence endocrine events that are often expressed over a period of hours to days, the subcutaneous morphine implants provide continuous opiate receptor stimulation over a similar time frame.

2. At physiologically relevant doses, the ability of morphine to enhance the LH inhibitory effects of T and E2 are more pronounced than those of DHT. Low circulating levels of DHT appear to reduce the LH secretory response to LHRH in preference to centrally inhibiting the release of LHRH.

It would appear that the feedback regulation of LH release in the male is orchestrated by several gonadal steroids acting at multiple loci within the hypothalamic-pituitary-LH axis. This supports past studies which indicate that E2 is important in the regulation of LH release in the male (D'Agata et al., 1981; P. Kalra and Kalra, 1981; Nishihara and Takahashi, 1983). The assumption that the E2 and DHT implants provided physiologically relevant doses is based on work done by others (P. Kalra and Kalra, 1980,

1981). Since E2 and DHT in the hypothalamus and DHT in the pituitary can be metabolized from T, it is not certain whether the actions of E2 and DHT are normally mediated via circulating levels of these steroids (Massa et al, 1972; Naftolin et al., 1975).

3. The interaction between chronic morphine and T in inhibiting LH release in male rats is not clearly reflected in changes in the metabolism of NE, DA, or 5HT in the MBH or POA-AH.

Of the three neurotransmitters evaluated, DA metabolism in the POA-AH was most responsive to morphine and T administration. An interaction between the two treatments was not apparent, however. These studies support evidence that T and the opiates are inhibitory to hypothalamic dopaminergic activity (Gudelsky and Porter, 1979; Simpkins et al., 1980), but do not support the notion that opiates exert their effects on the LHRH neuron by influencing DA activity (Rotsztejn et al., 1978).

While a large body of evidence suggests that NE stimulates LHRH output from the hypothalamus, and mediates opiate effects on LH release, the present data do not support this contention (S. Kalra and Simpkins, 1981; Van Vugt et al., 1981; Adler and Crowley, 1984). The differences between this and previous studies may be related to the chronic treatment paradigm employed in this work. While the measurement of NME levels provides a noninvassive

measure of noradrenergic activity, being present in low concentrations in hypothalamic tissue, NME may not be a sensitive index of noradrenergic activity. While more difficult to quantify, the NE metabolite, 3-methoxy-phenylethylene-glycol, in its free and conjugated forms, may be present in larger quantities and could serve as a better metabolic indication of NE release in the rat (Elchisak and Carlson, 1982).

4. The narcotic antagonist, naloxone, stimulates LH release at all times during the rat estrous cycle, including during the proestrous LH surge.

The mechanism through which naloxone stimulates LH release is likely through antagonizing the ongoing actions of EOP, since the application of appropriate EOP antibodies also stimulates LH release (Schulz et al., 1981, Forman et al., 1983). These studies clarify earlier evidence, using naloxone as an indication of opioid inhibition of LH release, that EOP are important in the maturation of the central mechanisms controlling LH release, but are not involved in regulating LH release in the adult female rat (Blank et al., 1979). Rather, it appears that EOP are important in maintaining basal LH release throughout the estrous cycle and perhaps in limiting the magnitude of the proestrous LH surge. A corollary to this finding is that when appropriately administered, opioid agonists and antagonists could modify the reproductive status of the animal (see below).

5. The ability of naloxone to stimulate LH release is reduced during the hypersecretion of LH induced by P administration to proestrous or EB-primed ovariectomized rats.

The ability of naloxone to stimulate LH release is very sensitive prior to the EBP-induced LH surge and during the EB-induced LH surge in ovariectomized rats. Minimal doses required to elicit LH secretion are comparable to doses needed to stimulate LH release in male rats (Cicero et al., 1981). It would appear that a considerable amount of EOP inhibition of LH release exists at these times in the female rat and possibly during the period of basal LH release in the estrous cycle.

In contrast, naloxone was relatively ineffective in stimulating LH release during the period of hypersecretion seen following the sequential administration of EB plus P to ovariectomized rats. Since doses of naloxone which elicited LH release at this time were more than sufficient to block any ongoing EOP activity at their receptors, it is safe to assume that opioid inhibition of LH release at this time was diminished

The effects of naloxone on LH hypersecretion yields information regarding the relation between gonadal steroid-induced LH hypersecretion in ovariectomized rats and the proestrous LH surge. The sequential administration of EB plus P to ovariectomized rats is in several respects an

appropriate experimental model for the proestrous LH surge. This includes the similarities in time course and magnitude of the two LH surges, and the temporal changes in hypothalamic tissue levels of LHRH and catecholamine activity associated with this hormonal event (P. Kalra and Kalra, 1977a; Lofstrom, 1977; Simpkins et al., 1979; S. Kalra and Kalra, 1979). However, LH hypersecretion can be elicited by E2 and P administered to ovariectomized rats at doses much lower than what are commonly used (McPherson and Mahesh, 1979). Also, the steroid milieu during and after the proestrous LH surge is not accurately reflected in this model. Consequently, some investigators have proposed that the LH surge induced by E2 or EB alone more precisely models the neuroendocrine changes that occur on proestrus (Barraclough and Wise, 1982). This model ignores the potential contribution of the increased P secretion that accompanies the proestrous LH surge (S. Kalra and Kalra, 1974a). While endogenous P levels on proestrus do not compare to levels achieved by an exogenous P injection, circulating steroid levels are not the only indication of the effectiveness of a biological response. Since E2 induces the synthesis of cytosolic P receptors (Rainbow et al., 1982), rats in their late follicular phase might be highly sensitive to changes in P titers.

In the present work, naloxone stimulated LH hypersecretion during the proestrous and ovariectomized EB-

induced LH surges, but was ineffective during LH hypersecretion in ovariectomized EBP-treated rats. In this respect, the LH surge in EB-treated rats more accurately reflects the inhibition of LH secretion by EOP seen during the proestrus LH surge. A decline in EOP inhibition was noted before the proestrous LH surge since as pituitary responsiveness to LHRH increases over the follicular phase, naloxone-induced LH secretion remains constant (Cooper et al., 1973; Aiyer et al., 1974; Gabriel et al., 1983; Chapter VII). It is probable that a decline in EOP inhibition of LH secretion may have occured during the proestrus LH surge that was not indicated through the use of naloxone as an index of EOP neuronal activity.

6. Chronic morphine treatment enhances both the inhibitory and stimulatory effects of E2 on gonadotropin release in ovariectomized rats.

This extends the studies initiated in the male by showing that morphine acts to stimulate both the negative and positive feedback actions of gonadal steroids in the female. These findings suggest that EOP are important in modulating the feedback actions of E2 in the female rat. It further lends support to the notion that EOP are involved in the advanced onset and increased magnitude of the LH surge seen following P treatment to proestrous and EB-treated ovariectomized rats, since morphine treatment acted in a similar fashion in these E2-treated animals. Because

morphine and EOP appear to exert both inhibitory and stimulatory effects on LH and FSH release, their role in regulating gonadotropin secretion is more complex than previously anticipated (Piva et al., 1984).

The work presented in this dissertation supports the contention that alterations in the activity of EOP neurons modify the sensitivity of the hypothalamus to gonadal steroids. This implicates EOP in the maintenance of reproductive endocrine homeostasis and in the regulation of basal and cyclic gonadotropin secretion. While these studies employed the pharmacologic application of an opioid agonist or antagonist to infer EOP neuronal activity, the measurement of EOP as an index of neuronal activity has not been adequately studied in the rat. Quantifying EOP levels in the hypophysial portal plasma or in a push-pull perfusate would presumably serve as an index of release. These techniques though difficult to perform, have been reported for other neuropeptides (Sarkar et al., 1976; Levine and Ramirez, 1982; Kasting et al., 1981). A low post-surgical success rate and an inevitable amount of tissue damage must be anticipated with these methods.

It is difficult to infer neuronal activity based solely on tissue levels of a neuropeptide. While ovariectomy does not appear to change beta-endorphin levels in the hypothalamus, prolonged E2 treatment decreases levels of this peptide in the hypothalamus in a manner reversed by P

(Wardlaw et al., 1982). Although the doses of steroid administered in this study were in the physiological range, inadequate data on hormone levels, time of sacrifice, and size of the tissue fragments makes this study difficult to interpret. Changes in EOP levels in the hypothalamus during the estrous cycle are also of interest. However, data on this topic are conflicting (Kumar et al., 1980; Wardlaw et al., 1982; Barden et al., 1981a; Knuth et al., 1984).

In contrast to the brain, EOP levels in the pituitary are more clearly responsive to changes in the steroid milieu. Beta-endorphin levels in the anterior pituitary decline after ovariectomy, and increase in the plasma and NIL on the afternoon of proestrus (Lee et al., 1980; Ishizuka et al., 1982). While this agrees with the present pharmacologic observations that EOP activity is altered after gonadectomy and during the periovulatory interval, opiates do not appear to influence gonadotropin secretion at the level of the pituitary Chapters IV, V and IX; Cicero et al., 1980b; Wiesner et al., 1984). It is possible that these hypophyseal EOP could influence gonadotropin secretion centrally through an uncharacterized hypophyseal pathway (Bergland and Page, 1979), or through an action of circulating EOP in the plasma. It has been suggested that EOP exert their actions by acting on circumventricular regions of the brain, such as the median eminence. (Panerai et al., 1983). While methionine-enkephalin likely cannot act

through a plasma route, beta-endorphin could (Bloom et al., 1978). Rather than being the cause of steroid-induced changes in gonadotropin secretion, these alterations in pituitary EOP levels likely reflect similar neural and hormonal influences.

There are similarities between the mode of EOP influece on gonadotropin secretion in humans and rodents. In adult men, naloxone stimulates LH secretion, indicating that EOP exert a tonic inhibition of LH secretion in males of both species (Delitalia et al., 1983). Additionally, the ability of opiates to influence LH secretion is diminished in castrated men, and is restored after gonadal steroid treatment (Foresta et al., 1983a,b). The rat, therefore may serve as an appropriate experimental model to study gonadal steroid interactions with opioids in the regulation of LH secretion in males.

In humans and nonhuman primates, cyclic changes in gonadotropin secretion may be more dependent on ovarian rather than neural signals. This is evidenced by the maintenance of menstrual cycles in monkeys in the presence of an LHRH stimulus which does not change in amplitude or frequency (Knobil, 1980). More recent work in human and nonhuman primates indicates that normal menstrual cyclicity does depend on an LHRH signal that varies in frequency and amplitude, and that EOP are important in modifying this signal (Ferin et al., 1984) In women, naloxone stimulates LH

secretion during the luteal and late follicular phases but not during the early follicular phase of the menstrual cycle (Quigley and Yen, 1980; Blankstein et al., 1981; Snowden et al., 1984). In primates, follicular phase LH secretion is characterized by high frequency low amplitude LH pulses, while luteal phase LH secretion is characterized by low frequency high amplitude LH pulses (Van Vugt et al., 1984). Although naloxone does not alter the pattern of LH secretion during the early follicular phase, the infusion of naloxone during the luteal phase of the menstrual cycle causes an increase in LH pulse frequency similar to that seen during the follicular phase of the menstrual cycle. Evidently, luteal P secretion modifies LH pulse amplitude by altering EOP activity. Overriding this luteal phase pattern of LH secretion with exogenously administered LHRH disrupts the menstrual cycle (Ferin et al., 1984). Finally, naloxone-induced LH release returns in the late follicular phase of the menstrual cycle near the preovulatory LH surge (Ferin et al., 1984). In primates as in rats, EOP neuronal activity appears to be influenced by P and activated during the periovulatory period.

It appears that hypothalamic beta-endorphin may be involved in mediating these opioid-induced changes in LH secretion during the menstrual cycle. Both P and E2 appear to influence beta-endorphin release into hypophyseal portal vessels of monkeys (Ferin et al., 1984). Following

menstruation or ovariectomy, beta-endorphin concentrations in portal plasma are undetectable (Wehrenberg et al., 1982). If beta-endorphin acts to inhibit LHRH output, then this would explain the inability of naloxone to stimulate LH release after luteolysis. Similarly, as E2 titers increase during late follicular development the reinitiation of beta-endorphin release would be evidenced by a resumption in naloxone-induced LH release. At this time ECP inhibition of LH release would aid in timing preovulatory LH release with follicular maturation.

The present work helps to understand the deleterious effects of opiate abuse on reproductive hormone secretion. Azizi et al. (1973) were among the first to present clinical evidence of depressed T levels in heroin addicts. In this investigation, depressed T levels were seen in the presence of normal LH secretion. Because opiates enhance the feedback sensitivity of the hypothalamus to gonadal steroids, it is reasonable to expect lower T levels to maintain normal LH output in these opiate dependent individuals. Similarly, the present work clarifies reports in female narcotic abusers (Gaulden et al., 1964). Since opiate receptor stimulation in females alters the magnitude and time course of the LH surge, an appropriately timed heroin injection could prevent preovulatory LH release. Prolonged opiate abuse could cause inappropriate LH hypersecretion prior to or after follicular maturation, leading to infertility and

improper luteal development. Additionally, it is of interest that some of the antigonadotropic actions of other CNS depressants may act through an opioid mechanism (Cicero et al., 1982b).

Both clinical and experimental work suggest that EOP may be important in seasonal, developmental, and pathological changes in reproductive hormone secretion. For example, in hamsters, exposure to short photoperiods is characterized by testicular degeneration or anestrus due to increased sensitivity to gonadal steroid feedback (Sisk and Turek, 1983). The altered opioid receptor concentrations associated with short days in hamsters might indicate opioid involvement in this increased gonadal steroid feedback (Wilkinson et al., 1983).

In a similar manner, increased sensitivity to gonadal steroid feedback has been noted in prepubertal and aged rats and after experimentally induced hyperprolactinemia (McCann et al., 1974; Gray et al., 1980; McNeilly et al., 1983). In all of these states, an alteration in EOP levels in the hypothalamus has been noted (Steger et al., 1980; Barden et al., 1981b; Forman et al., 1981; Facchinetti et al., 1983; Simpkins et al., 1984). It is of interest that since this project was initiated several clinical and experimental studies have suggested opioid involvement in the reproductive dysfunction associated with these states (Steger et al., 1980; Veldhuis et al., 1982; Reid et al., 1983; Carter et al., 1984; Simpkins et al., 1984).

If EOP participate in normal, developmental, and pathological changes in in gonadotropin secretion, then appropriately administered opiates should modify gonadotropin secretion in these states. Narcotic antagonists should, therefore, be able to modify the effects of altered EOP activity during reproductive senescence and hyperprolactinemia (Reid et al., 1983; Carter et al., 1984). Since, tolerance develops to the ability of naloxone to stimilate LH secretion (Owens and Cicero, 1981; Gabriel and Simpkins, 1983), the pattern in which an opiate antagonist is delivered is important. Similarly, an opiate agonist could stimulate opiate receptors in a period of depressed activity. In this case, the unwanted effects of the opiate on other systems not involved in hormone secretion, such as analgesia or respiration, must be considered.

In conclusion, these studies demonstrate that opiates interact with gonadal steroids in their regulation of gonadotropin secretion, by increasing the sensitivity of the hypothalamus to circulating gonadal steroids. This indicates that EOP are important in the modulation of gonadal steroid feedback in both males and females, and in the regulation of basal and cyclic gonadotropin release.

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BIOGRAPHICAL SKETCH

It was a cold and windy day in Anchorage when my parents brought me back to the igloo. I knew that I had to move somewhere warmer. Most of my childhood and adolescence was spent in beautiful suburban Hazelwood, Missouri. A vacation to Florida when I was 9 years old left a lasting impression. Assisted by an academic scholarship and several liberal Democratic education programs, I entered Washington University in St. Louis in the fall of 1975. My Artium Baccalarri in psychology was awarded in May, 1979. The next year was spent buying a stereo and applying to graduate school using the now famous Sun-Belt Strategy. I moved to Florida in August 1980 to meet my destiny.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of schclarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctcr of Philoscphy.

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